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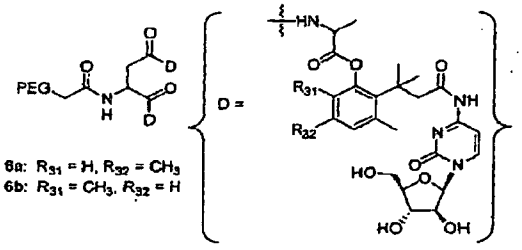
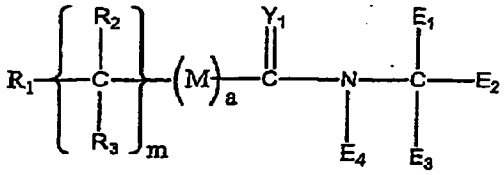
(54)【発明の名称】末端分枝高分子リンカーおよびそれを含む高分子複合体

(57)【要約】
多数のローディングが可能な末端分枝高分子プロドラッグプラットフォームを開示する。本発明の好ましい態様では、プロドラッグプラットフォームは活性物質を保持している各分枝がベンジル脱離反応を受けた後に多数の親化合物を放出する。プロドラッグの製造方法および哺乳類の治療におけるその使用方法もまた開示する。1つの好ましい態様では、式(I)などの高分子複合体を提供する。

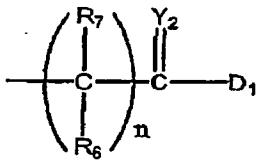


【特許請求の範囲】
【請求項１】
式：
【化１】

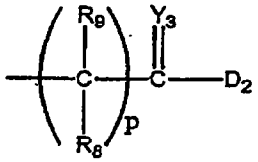
(I)



{式中、
R_1は高分子残基であり；
Y_1はO、SまたはNR$_4$であり；
MはO、SまたはNR$_5$であり；
E_1は
【化２】

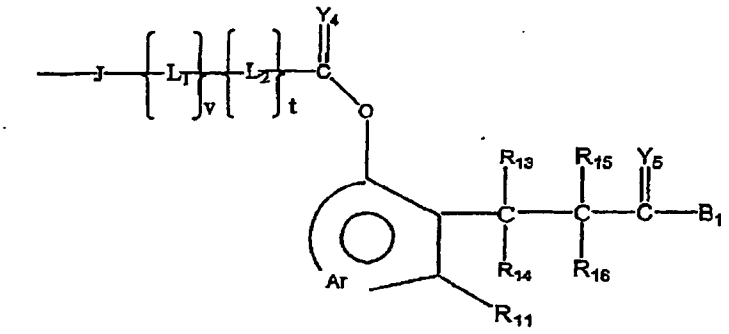


であり；
E_{2-4}は独立に、H、E_1または
【化３】

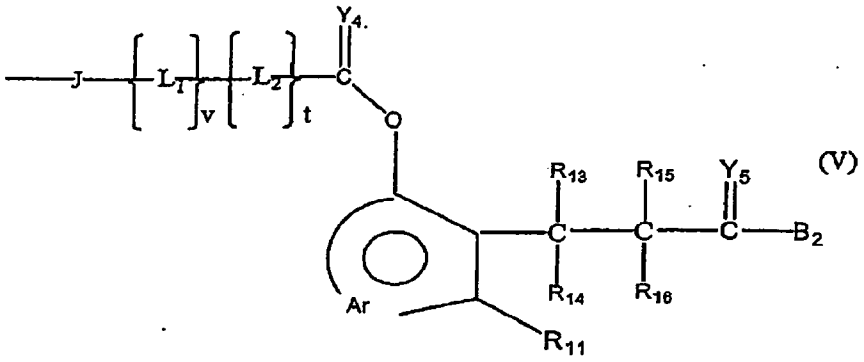


であり；
(a)は0または1であり；
(m)は0または正の整数であり；
(n)および(p)は独立に、0または正の整数であり；
Y_{2-3}は独立に、O、SまたはNR$_{10}$であり；
R_{2-10}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
D_1およびD_2は独立に、OH、

【化４】

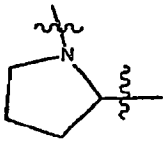


(IV)



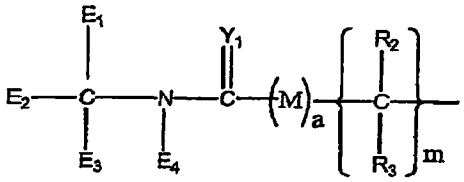
(V)

(式中、
(v)および(t)は独立に、0または約6までの正の整数であり；
JはNR$_{12}$または
【化５】



であり；
L$_1$およびL$_2$は独立に選択された二官能性リンカーであり；
Y$_{4-7}$は独立に、O、SおよびNR$_{14}$からなる群から選択され；
R$_{1-14}$は独立に、水素、C$_{1-6}$アルキル、C$_{3-12}$分枝鎖アルキル、C$_{3-8}$シクロアルキル、C$_{1-6}$置換アルキル、C$_{3-8}$置換シクロアルキル、アリール、置換アリール、アラルキル、C$_{1-6}$ヘテロアルキル、置換C$_{1-6}$ヘテロアルキル、C$_{1-6}$アルコキシ、フェノキシおよびC$_{1-6}$ヘテロアルコキシからなる群から選択され；
Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり；
B$_1$およびB$_2$は独立に、脱離基、OH、ヒドロキシル基含有成分またはアミン基含有成分の残基からなる群から選択される)
または末端分枝基である}
で表される化合物。
【請求項２】
R$_1$が水素、NH$_2$、OH、CO$_2$H、C$_{1-6}$基および

【化６】

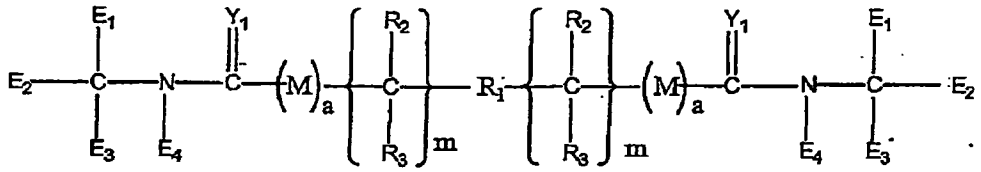


からなる群から選択されるキャッピング基Aをさらに含んでなる、請求項１に記載の化合物。

【請求項３】

式：

【化７】

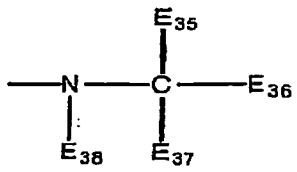


で表される、請求項２に記載の化合物。

【請求項４】

上記末端分枝基が式：

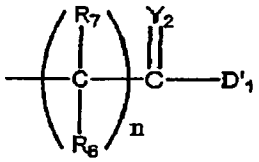
【化８】



[式中、

E_{35}は

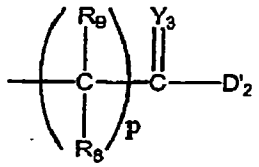
【化９】



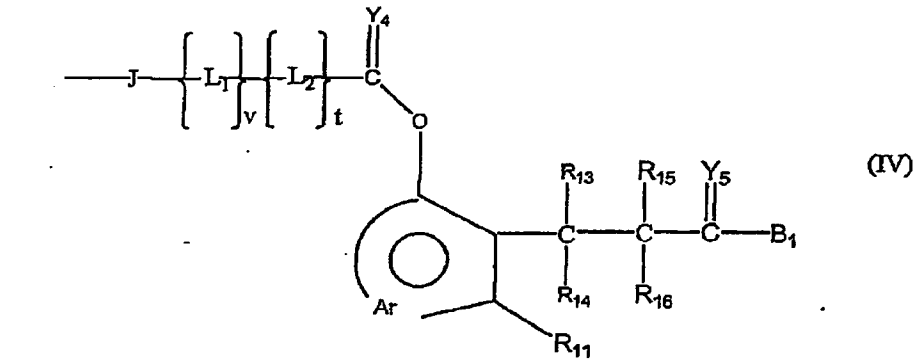
であり；

E_{36-38}は独立に、H、E_{35}または

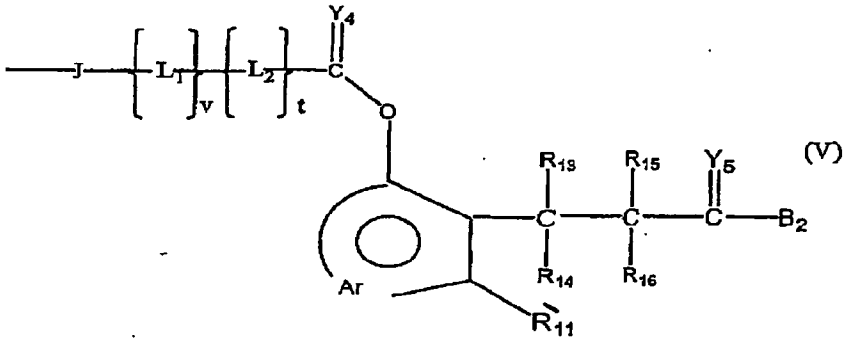
【化１０】



であり；
(n)および(p)は独立に、0または正の整数であり；
Y_{2-3}は独立に、O、SまたはNR_{10}であり；
R_{6-10}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
D'_1およびD'_2は独立に、OH、
【化１１】

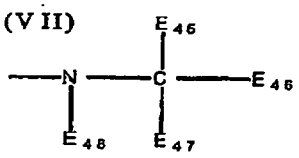


(IV)



(V)

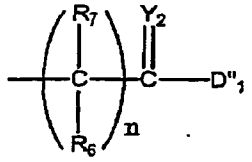
または
【化１２】



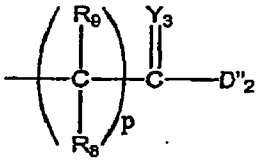
(VII)

{式中、
(v)および(t)は独立に、0または約6までの正の整数であり；
L_1およびL_2は独立に選択された二官能性リンカーであり；
Y_{4-7}は独立に、O、SおよびNR_{14}からなる群から選択され；
R_{11-14}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり；

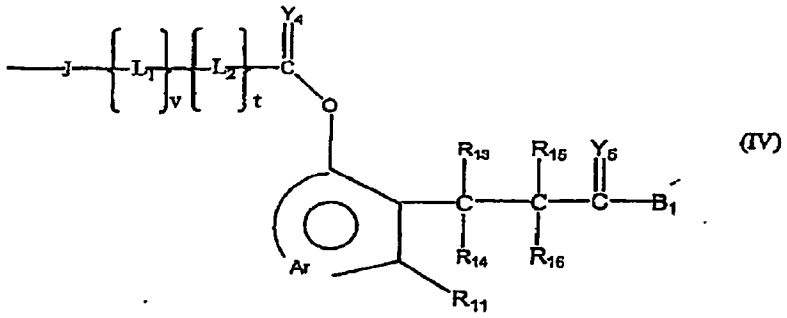
B_1およびB_2は独立に、脱離基、OH、ヒドロキシル基含有成分またはアミン基含有成分の残基からなる群から選択され；
E_{45}は
【化１３】



であり；
E_{46-48}は独立に、H、E_{45}または
【化１４】

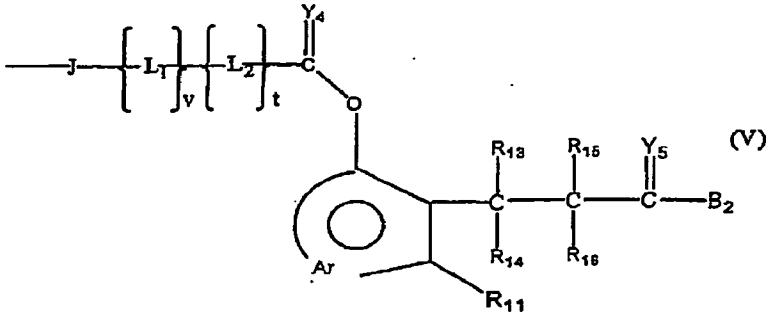


（式中、
D''_1およびD''_2は独立に、OH、
【化１５】



(IV)

または
【化１６】

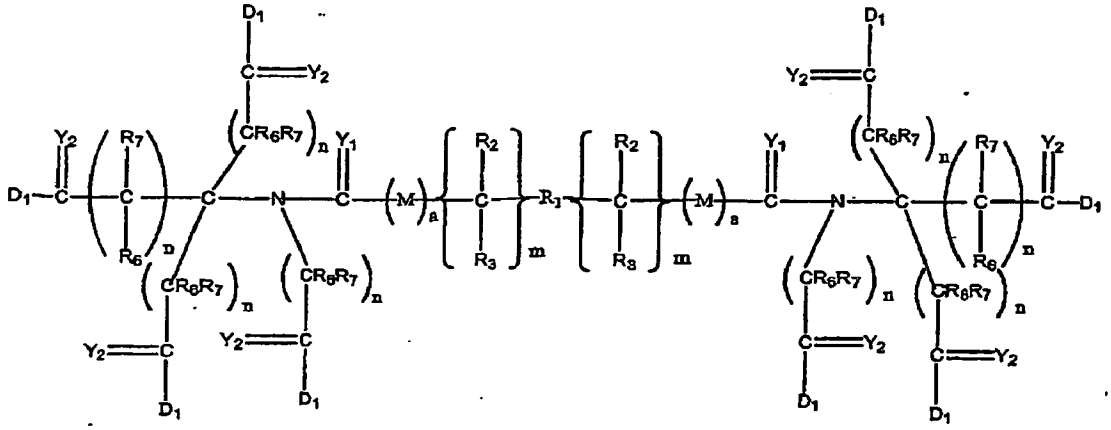


(V)

である）
である｝
である］

で表される、請求項１に記載の化合物。
【請求項５】
Y_1がOである、請求項３に記載の化合物。
【請求項６】
R_1がポリアルキレンオキシド残基を含んでなる、請求項１に記載の化合物。
【請求項７】
R_1がポリエチレングリコール残基を含んでなる、請求項６に記載の化合物。
【請求項８】
R_1がポリエチレングリコール残基を含んでなる、請求項３に記載の化合物。
【請求項９】
R_1が
$-C(=Y_6)-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-C(=Y_6)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-C(=Y_6)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-C(=Y_6)-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-C(=Y_6)-$、
$-C(=Y_6)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-Y_7-C(=Y_6)-$、
$-C(=Y_6)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{23}-C(=Y_6)-$、
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-O-(CR_{24}R_{25})_e-$、および
$-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{23}-$
(式中、
Y_6およびY_7は独立に、O、SまたはNR_{23}であり；
xは重合度であり；
R_{23}、R_{24}およびR_{25}は独立に、H、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
eおよびfは独立に、0、1、または2であり；かつ
Aはキャッピング基である)
からなる群から選択される、請求項６に記載の化合物。
【請求項１０】
R_1が$-O-(CH_2CH_2O)_x$を含んでなり、かつxは重量平均分子量が少なくとも約20,000であるような正の整数である、請求項９に記載の化合物。
【請求項１１】
R_1の重量平均分子量が約20,000～約100,000である、請求項３に記載の化合物。
【請求項１２】
R_1の重量平均分子量が約25,000～約60,000である、請求項３に記載の化合物。
【請求項１３】
式

【化１７】

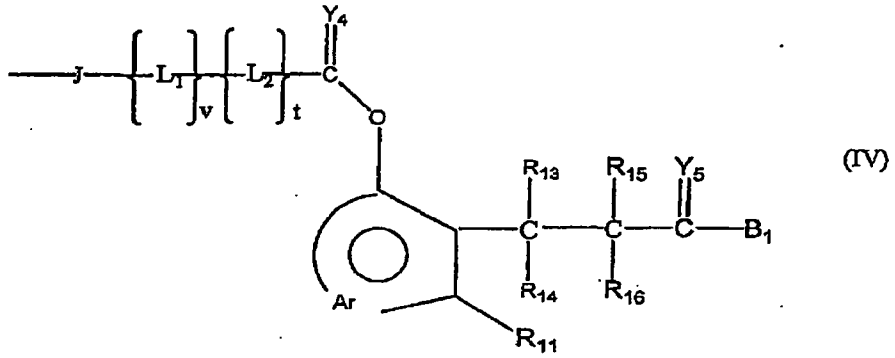


で表される、請求項３に記載の化合物。

【請求項１４】

D_1が

【化１８】



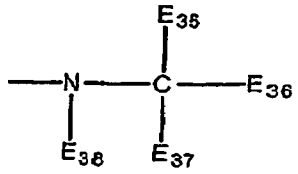
(IV)

である、請求項１３に記載の化合物。

【請求項１５】

D_1が

【化１９】



である、請求項１３に記載の化合物。

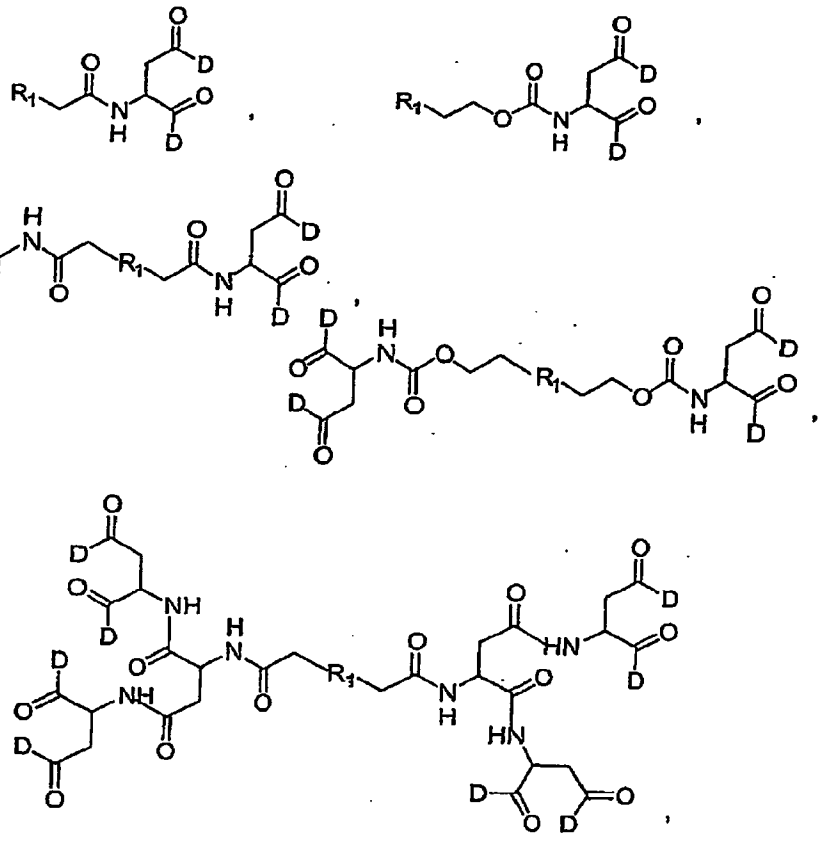
【請求項１６】

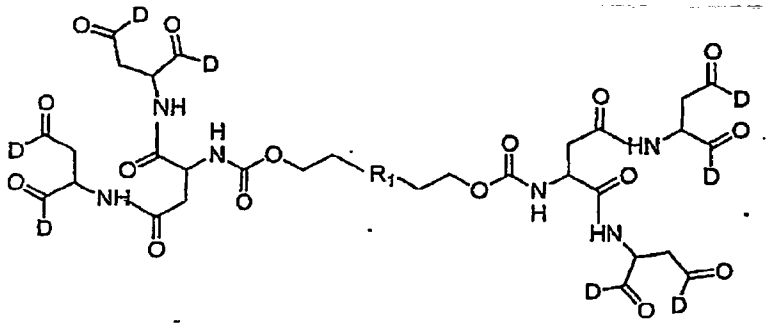
L_1が$(CH_2CH_2O)_2$である、請求項１に記載の化合物。

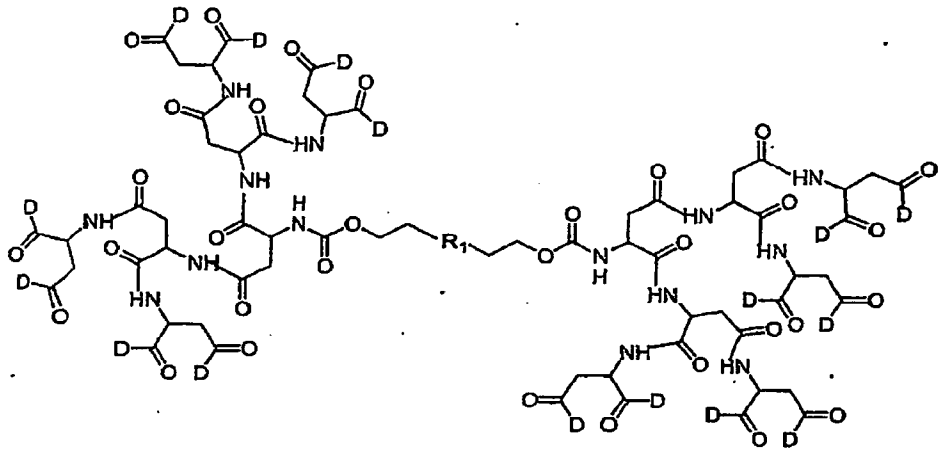
【請求項１７】

L_2が$-CH_2-$、$-CH(CH_3)-$、$-CH_2C(O)NHCH(CH_3)-$、$-(CH_2)_2-$、$-CH_2C(O)NHCH_2-$、$-(CH_2)_2-NH-$、$-(CH_2)_2-NH-C(O)(CH_2)_2NH-$および$-CH_2C(O)NHCH(CH_2CH(CH_3)_2)-$からなる群から選択され

る、請求項１に記載の化合物。
【請求項１８】
【化２０】

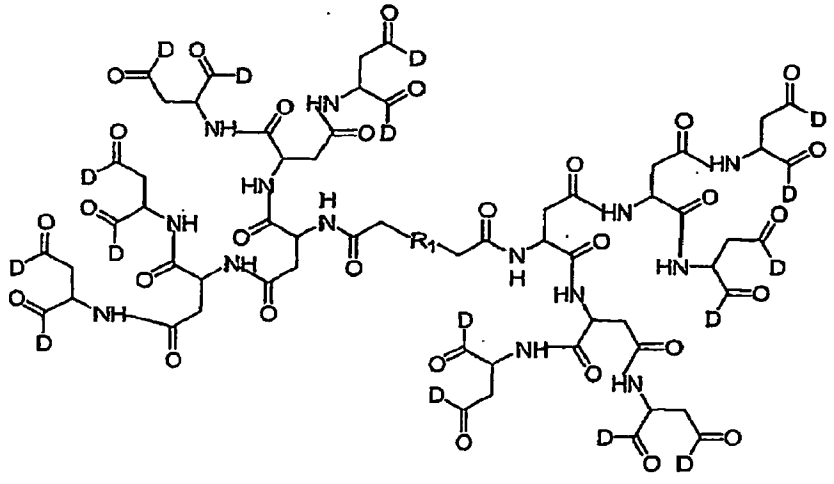






および

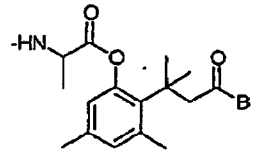
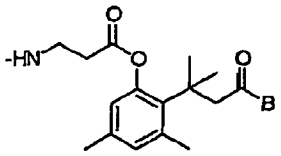
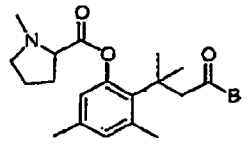
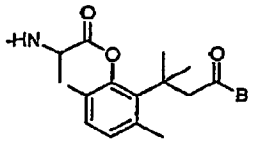
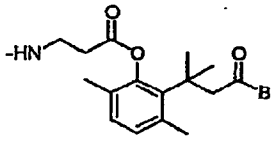
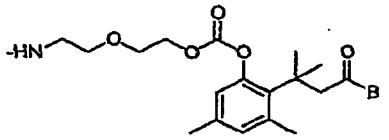
【化２１】



{式中、

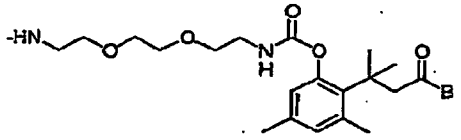
R_1はPEG残基であり、かつDは

【化２２】

および

【化２３】



(式中、
Bはアミンまたはヒドロキシル基含有薬物の残基である)
からなる群から選択される}
からなる群から選択される、請求項１に記載の化合物。

【請求項１９】

Bがダウノルビシン、ドキソルビシン；p-アミノアニリンマスタード、メルファラン、Ara-C(シトシンアラビノシド)、ロイシン-Ara-C、およびゲムシタビンからなる群のメンバーの残基である、請求項１８に記載の化合物。

【請求項２０】

治療が必要な哺乳類に、有効量の請求項１に記載の化合物(式中、D_1は生物学上活性な成分の残基である)を投与することを含んでなる、治療方法。

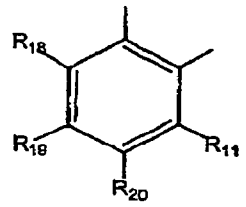
【請求項２１】

治療が必要な哺乳類に有効量の請求項１８に記載の化合物を投与することを含んでなる、治療方法。

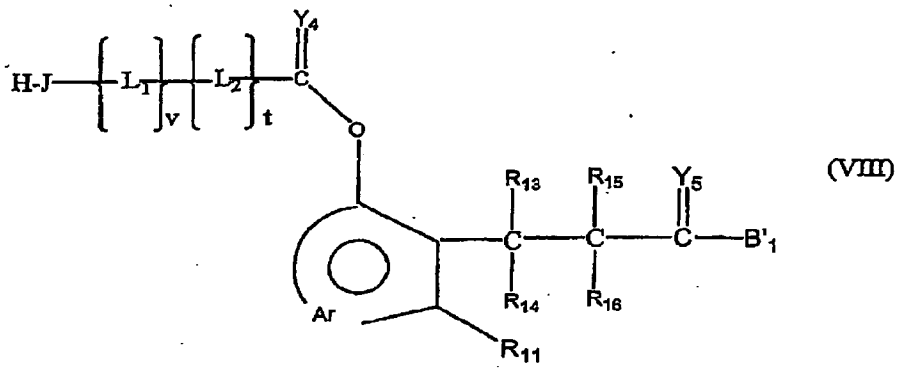
【請求項２２】

Arが式：

【化２４】

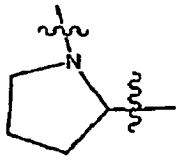


(式中、
R_{11}およびR_{18-20}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択される)
で表される、請求項１に記載の化合物。
【請求項２３】
R_{11}およびR_{18-20}が各々、HまたはCH$_3$である、請求項２２に記載の化合物。
【請求項２４】
高分子複合体の製造方法であって、
式(VIII):
【化２５】



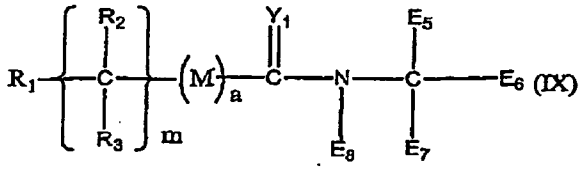
(VIII)

(式中、
(v)および(t)は独立に、0または約6までの正の整数であり;
JはNR_{12}または
【化２６】

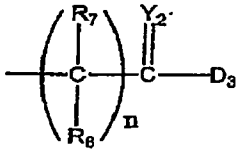


であり;
L_1およびL_2は独立に選択された二官能性リンカーであり;
Y_{4-5}は独立に、O、SおよびNR_{17}からなる群から選択され;
R_{11-17}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され;
Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分

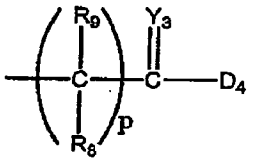
であり;かつ
B'_1はヒドロキシルまたはアミン基含有成分の残基である）
で表される化合物と、式(IX):
【化２７】



(式中、
E_5は
【化２８】



であり;
E_{6-8}は独立に、H、E_5または
【化２９】



であり;
D_3およびD_4は独立に、OH、保護されていないアミンまたはヒドロキシルと反応しうる脱離基、または末端分枝基であり;
R_1は高分子残基であり;
Y_1はO、SまたはNR$_4$であり,
Mは O、SまたはNR$_5$であり;
(a)は0または1であり;
(m)は0または正の整数であり;
(n)および(p)は独立に、0または正の整数であり;
Y_{2-3}は独立に、O、SまたはNR$_{10}$であり;かつ
R_{2-10}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択される）
で表される化合物とを、高分子複合体を生成させるのに十分な条件下で反応させることを含んでなる、上記方法。
【発明の詳細な説明】
【技術分野】
【０００１】
本発明は生物活性材料の長時間作用性複合体の作製に有用である新しいタイプの末端活性化高分子材料に関する。特に、本発明は治療的ペイロードの高い高分子系複合体およびその製造方法に関する。

【背景技術】
【０００２】
長年にわたり、生物学上有効な材料を哺乳類に投与するいくつかの方法が提案されてきた。多くの薬剤が水溶性塩として入手可能であり、比較的容易に医薬製剤に配合することができる。所望の薬剤が液体に不溶性である場合、またはin vivoで急速に分解される場合には問題が起こる。特に、アルカロイドは難溶である場合が多い。
【０００３】
薬剤を可溶性にする1つの方法がそれらを可溶性プロドラッグの一部として含める方法である。プロドラッグは投与した際にin vivoにおいて最終的に親化合物を遊離する生物学上活性な親化合物の化学誘導体を含む。プロドラッグは当業者によるin vivoにおける薬剤作用の発現および/または持続時間の改変を可能とし、体内での薬物の輸送、分配または溶解性を改変しうるものである。さらに、プロドラッグ製剤は毒性を減弱することも多く、あるいはまた医薬製剤を投与する場合に遭遇する問題を克服もする。プロドラッグの典型例としては、有機リン酸塩またはアルコールもしくはチオアルコールのエステルが挙げられる。Remington's Pharmaceutical Sciences, 16th Ed., A. Osol, Ed. (1980)を参照。なお、その開示は参照により本明細書に組み入れる。
【０００４】
プロドラッグは親化合物または活性化合物の生物学上不活性または実質的に不活性な形態である場合が多い。活性薬物の放出速度、すなわち、加水分解速度はいくつかの要因によって、特に、親薬物と改変剤とをつなぐ結合タイプにより影響を受ける。親化合物の十分な量の加水分解が起こる前に腎臓または細網内皮系などにより排出されるプロドラッグを製造することがないよう留意しなければならない。
【０００５】
高分子をプロドラッグ系の一部として組み込むことで薬物の循環寿命が長くなることが示されている。しかしながら、約10,000ダルトン未満の1種のみまたは2種の高分子各々とアルカロイド化合物などの特定の生物学上活性な物質とを複合体化した場合、特に、幾分耐加水分解性の結合が使用されている場合には、得られた複合体がin vivoで迅速に排出されることが分かっている。実際、かかる複合体は体から極めて迅速に排出されるため、加水分解が起こりやすいエステル結合が使用されている場合でさえも、治療効果に十分な親分子がin vivoにおいて再生されない。
【０００６】
カンプトテシンおよび生物学上活性な関連類似体は水溶性に乏しいことが多く、PEGプロドラッグ技術によって恩恵を得る物質の例である。当技術分野でのこれまでのいくつかの研究の概要を以下に示す。
【０００７】
Ohya, ら, J. Bioactive and Compatible Polymers Vol. 10 Jan., 1995, 51-66では、エステルをはじめとする種々の結合を介した2置換基の結合により作製されるドキソルビシン-PEG複合体を開示している。しかしながら、使用したPEGの分子量はせいぜい約5,000である。そのため、複合体は十分な結合の加水分解の前に実質的に排出されることから、in vivoにおける恩恵の実現は十分なものではない。
【０００８】
米国特許第4,943,579号では、水溶性プロドラッグとして塩形態の特定の単純な20(S)-カンプトテシンアミノ酸エステルを開示している。しかし、参考文献ではアルカロイドを比較的高分子量の高分子に結合させてプロドラッグを作製するための結合の一部としてアミノ酸を使用することについては開示されていない。表2で示された579人の患者に関するデータから明らかなように、加水分解は急速である。そのため、生理学的pHでは注入後に不溶性基剤が迅速に生じ、タンパク質と結合し、治療効果が達成される前に体から迅速に排出される。関連した取り組みは水溶性カンプトテシンナトリウム塩の開発に向けられた。
【０００９】
残念なことに、カンプトテシンの水溶性ナトリウム塩には依然として臨床応用に対する高

い有害性が残されていた(Gottlieb ら，1970 Cancer Chemother, Rep. 54, 461; Moertel ら，1972 上記，56, 95; Gottlieb ら，1972 上記，56, 103)。

【００１０】

本願出願人によるPCT公報WO96/23794では、1当量のヒドロキシル基含有薬物を高分子の各末端に結合したビス-複合体について記載している。このような進展があったものの高分子のペイロードをさらに高める技術が求められている。

【００１１】

このように、カンプトテシンおよび関連類似体などの治療成分からなるプロドラッグを作製するさらなる技術を提供する必要性がなお存在している。本発明ではこの必要性に取り組むものである。

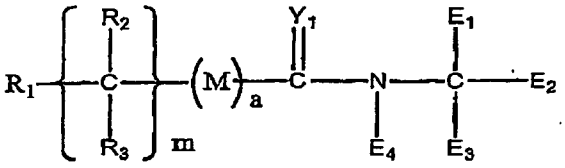
【発明の開示】

【００１２】

本発明の1つの態様では、式(I):

【化１】

(I)



【００１３】

(式中、

R_1 は高分子残基であり；

Y_1 はO、SまたはNR$_4$であり；

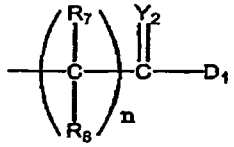
MはO、SまたはNR$_5$であり；

(m)は0または正の整数、好ましくは1または2であり；

(a)は0または1であり；

E_1 は

【化２】

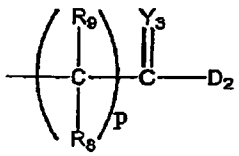


【００１４】

であり；

E_{2-4} は独立に、H、E_1 または

【化３】



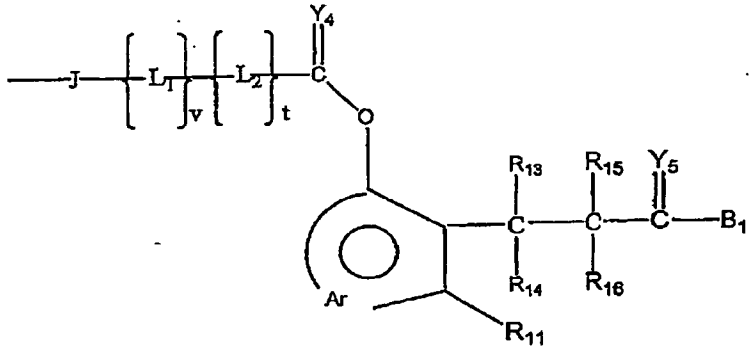
【００１５】

であり；

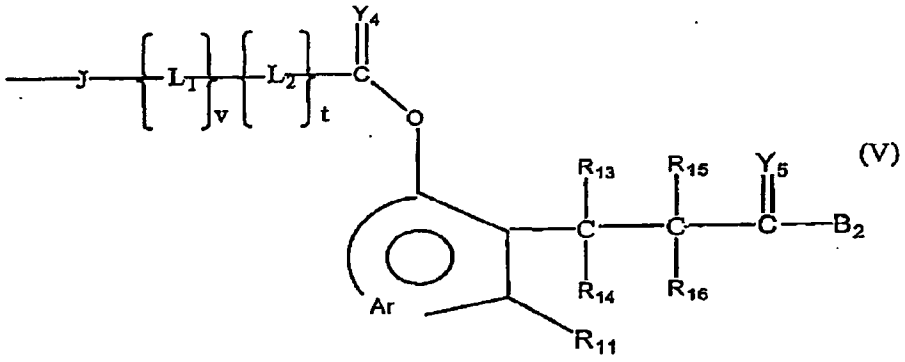
(n)および(p)は独立に、0または正の整数であり；

Y_{2-3} は独立に、O、SまたはNR$_{10}$であり；

R_{2-10}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
D_1およびD_2は独立に、OH、
【化４】

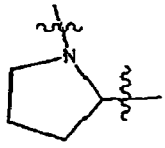


(IV)



(V)

【００１６】
または以下に示すさらなる末端分枝基である）
で表される化合物が提供される。
【００１７】
式(IV)および(V)中、
(v)および(t)は独立に、0または約6までの正の整数、好ましくは約1であり；
JはNR_{12}または
【化５】



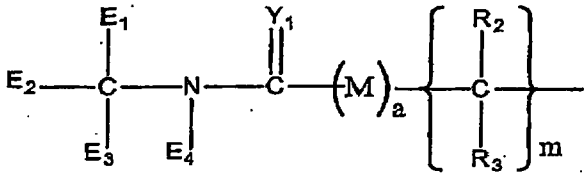
【００１８】
であり；
L_1およびL_2は独立に選択された二官能性リンカーであり；
Y_{4-5}は独立に、O、SおよびNR_{17}からなる群から選択され；
R_{11-17}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテ

ロアルコキシからなる群から選択され;
Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり;かつ
B_1およびB_2は独立に、脱離基、OH、ヒドロキシルまたはアミン基含有成分の残基からなる群から選択される。

【００１９】
本発明の1つの特に好ましい態様では、高分子残基の末端部が次の式(II):

【化６】

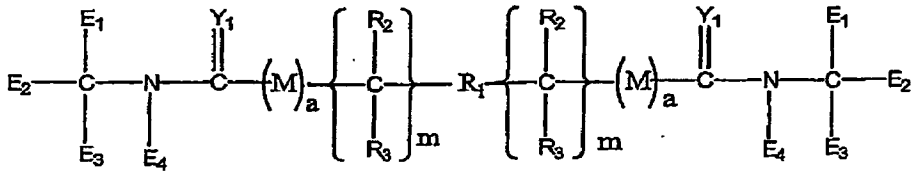
(II)



【００２０】
(式中、
全ての置換基および変数はこれまでに定義したとおりである)
で表される成分でさらに置換されている。よって、二官能性化合物は、本明細書においてB_1またはB_2とよばれる、2、4またはそれ以上の当量の生物学上活性な薬剤、薬物、またはタンパク質が送達されうるように高分子残基(R_1)がαおよびω両方の末端結合基を含有する場合に形成される。かかる二官能性高分子輸送形態の例は次の式(III):

【化７】

(III)



【００２１】
(式中、
全ての置換基および変数は上記のとおりである)
のように示される。

【００２２】
本発明の目的における「残基」とは、生物学上活性な化合物がプロドラッグ担体部分を結合するための置換反応を受けた後にも残存する生物学上活性な化合物の部分を意味するものとする。

【００２３】
本発明の目的における「アルキル」とは、直鎖、分枝鎖、置換（例えば、ハロー、アルコキシー、およびニトロー）C_{1-12}アルキル、C_{3-8}シクロアルキルまたは置換シクロアルキルなどを包含するものとする。

【００２４】
本発明の目的における「置換」とは、官能基または化合物に含まれる1個以上の原子に1個以上の異なる原子を付加するまたはそれと置き換えることを包含するものとする。

【００２５】
本発明の目的において、「置換アルキル」とは、カルボキシアルキル、アミノアルキル、ジアルキルアミノ、ヒドロキシアルキルおよびメルカプトアルキルを包含し;「置換シクロアルキル」とは、4-クロロシクロヘキシルなどの基を包含し;「アリール」とは、ナフチルなどの基を包含し;「置換アリール」とは、3-ブロモフェニルなどの基を包含し;「アラルキル」とは、トルイルなどの基を包含し;「ヘテロアルキル」とは、エチルチオフ

ェンなどの基を包含し；「置換ヘテロアルキル」とは、3−メトキシ−チオフェンなどの基を包含し；「アルコキシ」とは、メトキシなどの基を包含し；および「フェノキシ」とは、3−ニトロフェノキシなどの基を包含する。ハロ−はフルオロ、クロロ、ヨードおよびブロモを包含するものとする。

【００２６】

本発明の目的における「十分な量」とは、当業者によって理解される治療効果を達成する量を意味するものである。

【００２７】

本発明の化合物の最も重要な利点の1つはプロドラッグの高分子単位当たりのペイロードがこれまでの技術よりも高いことである。一般的には、高分子がまず加水分解によりトリメチルロック(TML)系プロドラッグ中間体を放出し、次いで得られた中間体または「第2のプロドラッグ」部分がラクトン化を受けて、例えば、生物学上活性な化合物またはさらなるプロドラッグを含んでなる組成物(composition)のいずれかである成分を再生することが好ましい。よって、本発明の高ペイロード高分子複合体は最大4つまでまたはそれ以上の数の薬物分子を含有しうる独自の送達系である。

【００２８】

本明細書において記載する化合物および複合体の製造および使用方法も提供される。

【００２９】

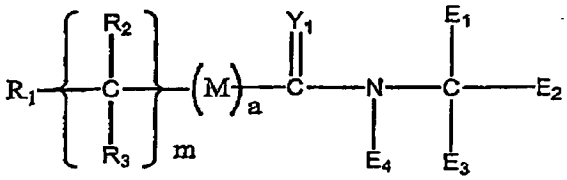
発明の詳細な説明

A.式 (I)

本発明の1つの好ましい実施形態では、式:

【化８】

(I)



【００３０】

{式中、

R_1 は高分子残基であり；

Y_1 はO、SまたはNR$_4$であり；

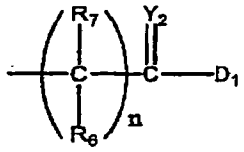
MはO、SまたはNR$_5$であり；

(a)は0または1であり；

(m)は0または正の整数であり；

E_1 は

【化９】

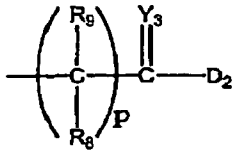


【００３１】

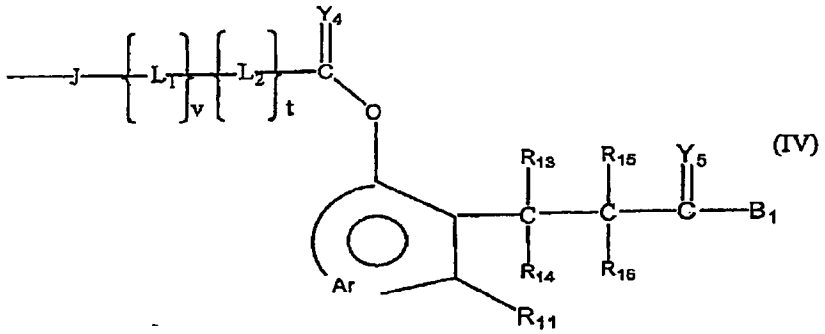
であり；

E_{2-4} は独立に、H、E_1 または

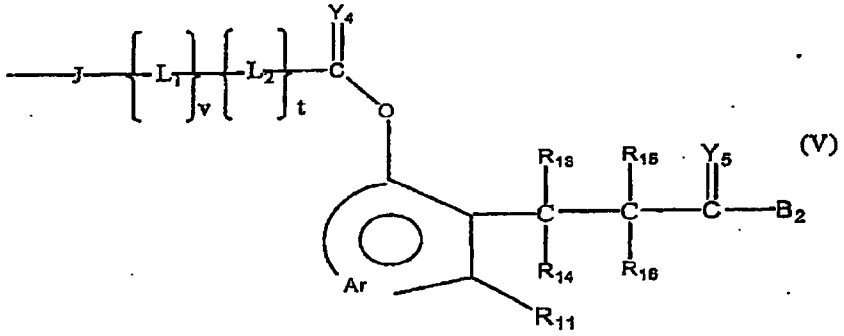
【化１０】



【００３２】
であり；
(n)および(p)は独立に、0または正の整数であり；
Y_{2-3}は独立に、O、SまたはNR_{10}であり；
R_{2-10}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
D_1およびD_2は独立に、OH、
【化１１】

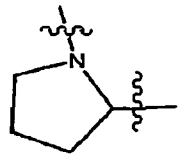


(IV)



(V)

【００３３】
(式中、
JはNR$_{12}$または
【化１２】



【００３４】
であり；

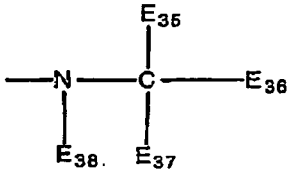
(v)および(t)は独立に、0または約6までの正の整数、好ましくは約1であり；
L_1およびL_2は独立に選択された二官能性リンカーであり；
Y_{4-5}は独立に、O、SおよびNR_{17}からなる群から選択され；
R_{11-17}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり；かつ
B_1およびB_2は好ましくは独立に、脱離基、OH、ヒドロキシル基含有成分またはアミン基含有成分の残基からなる群から選択される）
である}
で表される化合物が提供される。

【００３５】

もう1つの好ましい実施形態では、D_1およびD_2は独立に、式(VI)

【化１３】

(VI)

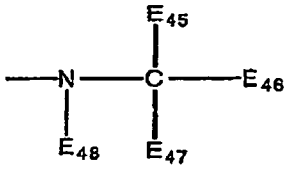


【００３６】

(式中、
E_{35-38}は定義内でD_1およびD_2が以下で定義するD'$_1$およびD'$_2$に変わることを除き、上記のE_{1-4}の定義と同じ基から選択される）
で表される選択された末端分枝基である。この実施形態では、D'$_1$およびD'$_2$が独立に、OH、式(IV)または(V)で表される成分、または

【化１４】

(VII)



【００３７】

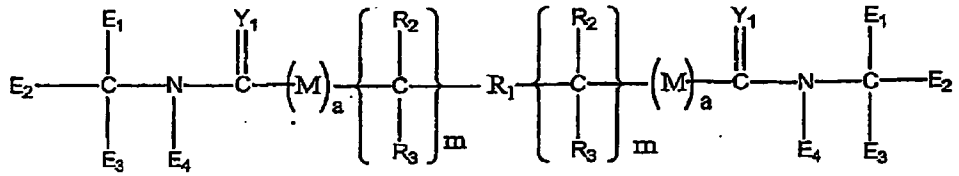
(式中、
E_{45-48}は定義内でD_1およびD_2がD''$_1$およびD''$_2$に変わり、かつD''$_1$およびD''$_2$が独立に、OH、式(IV)または式(V)であることを除き、E_{1-4}の定義と同じ基から選択される）
で表される成分でありうる。上記のことからわかるように、末端分枝がその最大限に二官能性高分子R_1を受け入れるとすると、最大16当量の薬物が高分子プラットフォームにロード(load)できる。

【００３８】

ビス-置換高分子残基が望まれるこの実施形態の態様では、本発明のいくつかの好ましい高分子輸送系が次の式

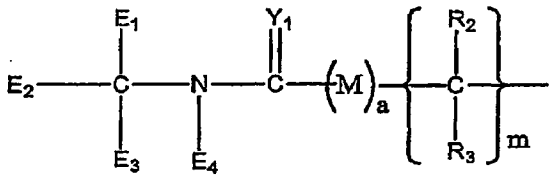
【化１５】

(III):



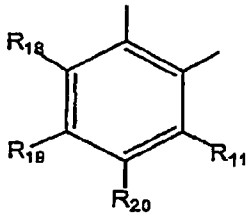
【００３９】
(式中、
全ての置換基および変数はこれまでに記載したとおりである)
のように示される。
【００４０】
本発明のマルチ・ローディング(multi-loading)高分子輸送系は主として本明細書においてR1とよばれる高分子残基に基づくものである。所望により、R1がキャッピング基Aを含有していてもよい。高分子キャッピング基Aとしては、例えば、水素、CO_2H、C_{1-6}アルキル基、およびビス系を形成する以下に示す式(II)の化合物などの成分が挙げられる:
【化１６】

(II)



【００４１】
(式中、
全ての置換基および変数はこれまでに記載したとおりである)。上記の多数の末端分枝がビス系においても等しく適用されることが分かるであろう。
【００４２】
本発明の式が含んでなるその他の置換基および変数に関しては、以下のものが好ましい:
Y_{1-5}は各々、酸素であり;
R_{2-10}およびR_{12}は各々、好ましくは水素または低級アルキル、例えば、C_{1-6}であり;
R_{11}、R_{13}およびR_{14}は好ましくは$-CH_3$であり;
(m)は1または2であり;
(n)および(p)は各々、0または1～4の整数のいずれかであり;
(v)は0または1であり;
(t)は1であり;
L_1は$-(CH_2CH_2O)_2-$であり; かつ
L_2は$-CH_2-$、$-CH(CH_3)-$、$-(CH_2)_2-$、$-(CH_2)_2-NH-$、$-CH_2C(O)NHCH(CH_3)-$、$-(CH_2)_2-NH-$、$-CH_2C(O)NHCH_2-$、$-(CH_2)_2-NH-C(O)(CH_2)_2NH-$または$-CH_2C(O)NHCH(CH_2CH(CH_3)_2)-$の1つである。
【００４３】
B.Ar成分の説明
式(I)に関して、Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であると考えられる。重要な特徴はAr成分が本質的に芳香性であることである。一般に、芳香族であるには、環分子面の上下にある「雲」内でπ電子が共有される必要がある。さらに、π電子数はヒュッケル則(4n+2)に従うものでなければならない。当業者ならば、無数の成分がその成分の芳香族必要条件を満たし、それゆえ本発明における使用に好適であることが分かるであろう。1つの特に好ましい芳香族基は:

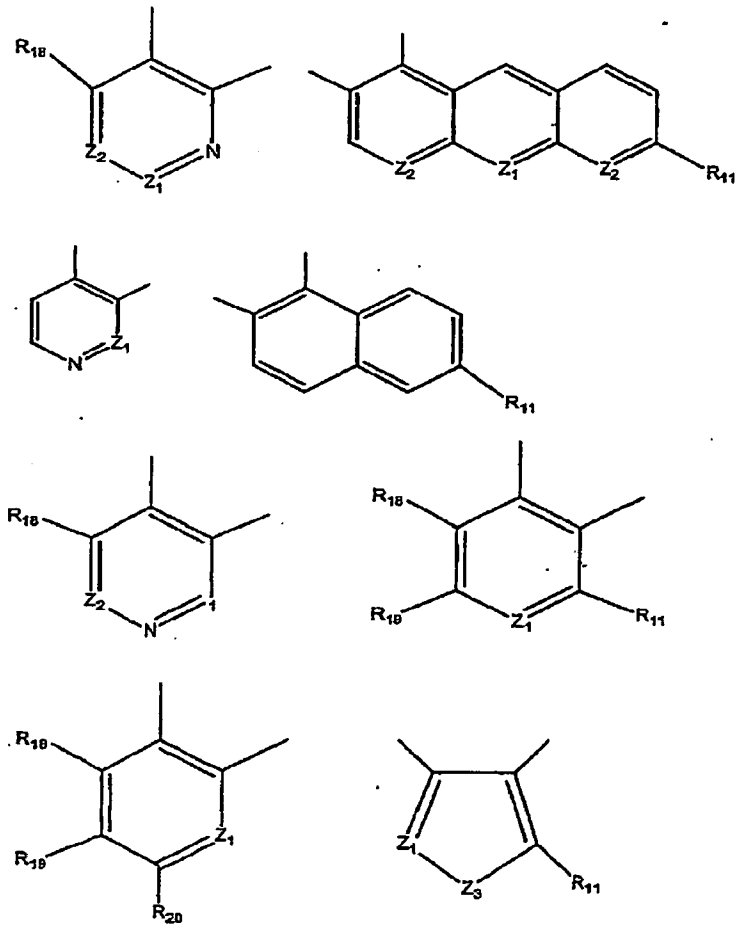
【化１７】



【００４４】

(式中、
R_{18-20}はR_{11}の定義と同じ基から選択される)
である。その他の芳香族基としては:

【化１８】



【００４５】

(式中、
Z_1およびZ_2は独立に、CR_{22}またはNR_{21}であり；かつ
Z_3はO、SまたはNR_{21}
(式中、
R_{18-22}はR_{11}の定義と同じ基またはシアノ、ニトロ、カルボキシル、アシル、置換アシルもしくはカルボキシアルキルから選択される)

である}
が挙げられる。ベンゾおよびジベンゾ系のほか、5および6員環を有する異性体もまた包含され、それらの関連同族体もまた包含される。また当業者ならば、ヒュッケル則に従いさえすれば、所望により、芳香環が0、S、NR_{21}などのヘテロ原子で置き換えられてもよいことが分かるであろう。さらに、所望により、芳香族または複素環式構造が当技術分野で一般的に理解されているハロゲンおよび/または側鎖で置き換えられてもよい。また、本発明のAr成分に好適な全ての構造はB_1またはB_2含有基および(R_{11})基を同一平面のオルト位に存在させることが可能である。

【0046】

C.プロドラッグの加水分解による薬物生成

本発明のプロドラッグ化合物は、血漿中の加水分解$t_{1/2}$が排出$t_{1/2}$よりも短くなるようにように設計される。

【0047】

治療を受けている哺乳類の血漿中における本発明の化合物に含まれる結合の加水分解$t_{1/2}$は、排出前に、十分な量の親化合物（すなわち、アミノまたはヒドロキシル基含有生物活性化合物）を放出させるのに十分短い加水分解$t_{1/2}$である。本発明のいくつかの好ましい化合物の血漿中における加水分解の$t_{1/2}$は約5分～約12時間の範囲である。好ましくは、組成物の血漿加水分解$t_{1/2}$が約0.5～約8時間の範囲、さらに最も好ましくは約1～約6時間の範囲である。

【0048】

D.実質的に非抗原性である高分子

上記のように、R_1はポリアルキレンオキシド(PAO)またはポリエチレングリコール(PEG)などの好ましくは実質的に非抗原性である水溶性高分子残基である。本発明の好ましい態様では、R_1は本明細書においてAとよばれる、二官能性またはビス-高分子系を形成しうる上記のキャッピング基をさらに含む。

【0049】

例として、本発明の組成物のPEG残基部分は、限定されるものではないが、次の:

$-C(=Y_6)-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-C(=Y_6)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-C(=Y_6)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-C(=Y_6)-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-C(=Y_6)-$、
$-C(=Y_6)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-Y_7-C(=Y_6)-$、
$-C(=Y_6)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{23}-C(=Y_6)-$、
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-O-(CR_{24}R_{25})_e-$、および
$-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{23}-$

(式中、
Y_6およびY_7は独立に、0、SまたはNR_{23}であり；
xは重合度であり；
R_{23}、R_{24}およびR_{25}は独立に、H、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
eおよびfは独立に、0、1、または2であり；かつ
Aはキャッピング基である)
から選択されうる。

【0050】

高分子の重合度(x)は約10～約2,300でありうる。これは高分子鎖の繰り返し単位数を示すものであり、高分子の分子量に依存している。(A)部分は本明細書において記載するキャ

ッピング基、すなわち、高分子の末端に見られる基であり、いくつかの態様では、H、NH_2、OH、CO_2H、C_{1-6}アルキルまたは当業者によって理解されているその他のPEG末端活性化基のいずれかから選択されうる。

【００５１】

また、ポリプロピレングリコール、本願出願人による米国特許第5,643,575号で記載されたものなどの分枝PEG誘導体、Shearwater Polymers, Inc. カタログ "Polyethylene Glycol Derivatives 1997-1998"で記載されたものなどの「星型PEG」および分岐したPEGも有用である。なお、上記の各々の開示は参照により本明細書に組み入れる。要すれば、過度の試験を行うことなく、二官能性結合基との結合のために水溶性高分子を官能化しうることが分かるであろう。

【００５２】

さらなる実施形態では、R_1は所望により、1種以上のデキストラン、ポリビニルアルコール、炭水化物系高分子、ヒドロキシプロピルメタクリルアミド、ポリアルキレンオキシドおよび/またはそのコポリマーから選択されてもよい。本願出願人による米国特許第6,153,655号も参照されたい。なお、その内容は参照により本明細書に組み入れる。

【００５３】

本発明の多くの態様では、複数置換高分子複合体が望まれる場合にはビス-活性化ポリエチレングリコールが好ましい。また、一置換高分子が望まれる場合にはポリエチレングリコール(PEG)、モノメチル基を末端にもつポリエチレングリコール(mPEG)などのモノ活性化C_{1-4}アルキル基を末端にもつポリアルキレンオキシド(PAO)が好ましい。

【００５４】

所望の加水分解可能な結合を提供するためには、モノまたはジPEGアミンおよびモノまたはジPEGジオールのほか、PEG酸またはPEG二酸などの一または二酸活性化高分子も使用できる。好適なPAO酸はまずmPEG-OHをエチルエステルに変換し、その後、鹸化することにより合成できる。Gehrhardt, H., ら、Polymer Bulletin 18: 487 (1987)およびVeronese, F. M., ら, J. Controlled Release 10; 145 (1989)も参照されたい。また、PAO酸はmPEG-OHをt-ブチルエステルに変換し、その後、酸開裂することにより合成できる。例えば、本願出願人による米国特許第5,605,976号を参照されたい。なお、上記の各々の開示は参照により本明細書に組み入れる。

【００５５】

PAOおよびPEGは平均分子量の点で実質的に異なりうるが、本発明のほとんどの態様においてプロドラッグの高分子部分の重量平均分子量は少なくとも約20,000である。好ましくは、R_1の重量平均分子量は約20,000～約100,000、さらにより好ましくは約25,000～約60,000である。プロドラッグに含有させるのに選択される高分子の平均分子量は、リンカーの加水分解前に、プロドラッグの十分な循環を提供するのに十分なものでなければならない。

【００５６】

本明細書において包含される高分子物質は、好ましくは室温で水溶性である。限定されるものではないが、かかる高分子としては、ポリエチレングリコール(PEG)またはポリプロピレングリコールなどのポリアルキレンオキシドホモポリマー、ポリオキシエチレン化ポリオール、およびそのコポリマー、ならびにブロックコポリマーの水溶性が維持される場合にはそのブロックコポリマーが挙げられる。

【００５７】

PEGなどのPAOについて本明細書において記載したように同様の活性化が行われるなら、デキストラン、ポリビニルアルコール、炭水化物系高分子、ヒドロキシプロピルメタクリルアミド(HPMA)、およびそのコポリマーなどのような有効に非抗原性な材料をPAO系高分子の代わりとして使用できる。当業者ならば、上記のリストは例示にすぎず、本明細書において記載する性質を有する全ての高分子材料が包含されることが分かるであろう。本発明の目的では、「有効に非抗原性」および「実質的に非抗原性」とは、当技術分野において実質的に毒性がなく、かつ哺乳類において感知できる免疫応答を誘導しないと認識される

全ての高分子材料を包含するものと理解される。

【００５８】

ポリプロピレングリコール酸など上記のもの以外のポリアルキレンオキシド誘導体、ならびにその他の二官能性結合基もまた包含されることは上記の説明から明らかであろう。

【００５９】

E.プロドラッグ候補

1.ヒドロキシル基含有化合物の残基

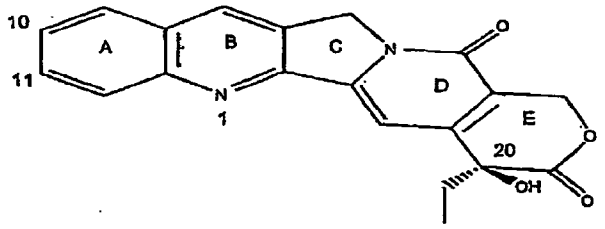
a.カンプトテシンおよび関連トポイソメラーゼI阻害剤

カンプトテシンは中国で自生するカンプトテカ・アクミナタ(Camptotheca accuminata)の樹木およびインドで自生するクサミズキ(Nothapodytes foetida)の樹木で産生される水に不溶性の細胞傷害性アルカロイドである。カンプトテシンおよび関連化合物ならびに類似体は有望な抗癌または抗腫瘍剤であることも知られており、さらにこれらの活性がin vitroおよびin vivoにおいて発揮されることも分かっている。また、カンプトテシンおよび関連化合物は本発明のプロドラッグへの変換候補でもある。

【００６０】

カンプトテシンおよび特定関連類似体は共通した構造：

【化１９】



【００６１】

を有している。

【００６２】

この主要構造から、いくつかの公知な類似体が製造されてきた。例えば、A環はOHで10および11位のいずれかまたはその両方を置換しうる。また、A環は直鎖または分枝鎖C_{1-30}アルキルまたはC_{1-17}アルコキシ(所望により、ヘテロ原子、すなわち、OまたはSで環と結合していてもよい)で9位も置換しうる。B環は直鎖もしくは分枝鎖C_{1-30}アルキルもしくは置換アルキル、C_{5-8}シクロアルキル、C_{1-30}アルコキシ、フェニルアルキルなど、アルキルカルバメート、アルキルカルバジド、フェニルヒドラジン誘導体、アミノ、アミノアルキル、アラルキルなどで7位を置換しうる。C、DおよびE環でもその他の置換が可能である。例えば、米国特許第5,004,758号；第4,943,579号；第Re 32,518号を参照されたい。その内容は参照により本明細書に組み入れる。かかる誘導体は過度な試験を行わなくとも、公知の合成方法を用いて作製できる。本発明における使用に好ましいカンプトテシン誘導体としては、20-OHまたは本明細書において記載する活性化型高分子輸送系と直接反応しうるまたは後にPEGなどの高分子と結合する結合部分中間体、例えば、イミノ二酢酸などと反応しうるもう1つのOH基を含むものが挙げられる。本明細書において記載したカンプトテシン類似体は例示を目的とするものであって、これに限定されない。

【００６３】

b.タキサン系化合物およびパクリタキセル誘導体

本発明のプロドラッグ組成物に含められる化合物種の1つがタキサン系化合物である。本発明の目的では、「タキサン」とは、タキサン系テルペンに入る全ての化合物を包含するものである。よって、タキソール(パクリタキセル)、3'-置換 tert-ブトキシ-カルボニル-アミン誘導体(タキソテール)など、ならびに標準有機技術を用いて容易に合成されるまたはSt. Louis, MissouriのSigma Chemicalなどの民間供給会社から入手可能であるその他の類似体は本発明の範囲である。これらの誘導体は有効な抗癌剤であることが分かって

いる。多くの研究により、これらの薬剤が数種類の悪性腫瘍に対する活性を有することが示されている。現在まで、それらの使用には、特に、それらの供給が不足しており、水溶性が乏しく、さらに過敏症を引き起こす傾向があることから厳しい制限があった。本願出願人による米国特許第5,622,986号および第5,547,981号で開示された7-アリール-カルバメートおよび7-カルバザートをはじめとするその他のタキサン系化合物もまた本発明のプロドラッグに含めうることは理解すべきである。なお、上記米国特許の内容は参照により本明細書に組み入れる。パクリタキセルは好ましいタキサンである。

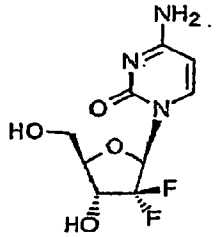
【００６４】

c.さらなる生物活性成分

上記分子のほか多くの化合物を用いて本発明のプロドラッグ製剤が製造できる。例えば、ビス-PEG複合体などの生物学上活性な化合物が、

ゲムシタビン：

【化２０】

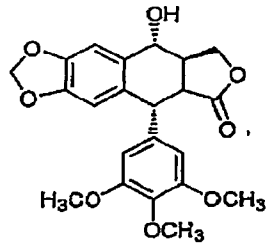


【００６５】

または

ポドフィロトキシン：

【化２１】

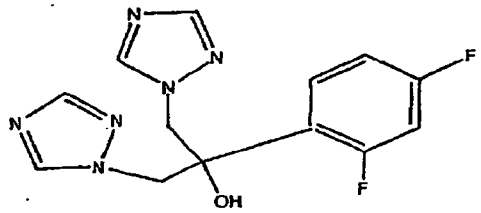


【００６６】

または

フルコナゾールなどのトリアゾール系抗真菌薬：

【化２２】

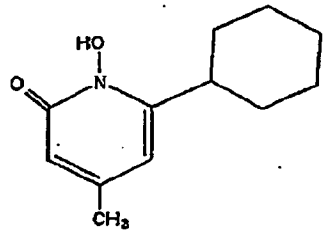


【００６７】

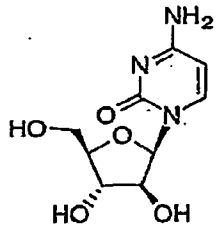
または

シクロピロックス：

【化２３】



【００６８】
または
Ara-C:
【化２４】



【００６９】
などの化合物から誘導された。
【００７０】
本発明の高分子系プロドラッグは特にかかる水不溶性化合物を送達するのに十分に好適なものであるが、プロドラッグ形態用に選択される親化合物が実質的に水不溶性である必要はない。その他の有用な親化合物としては、例えば、生物学上活性な特定の低分子量タンパク質、酵素およびペプチドグリカンをはじめとするペプチド、ならびにその他の抗腫瘍剤；フォルスコリンなどの心血管作動薬；コンブレタスタチン、ビンブラスチン、ドキソルビシン、メイタンシンなどの抗新生物薬；バンコマイシン、エリスロマイシンなどの抗感染症薬；ナイスタチン、アムホテリシンB、トリアゾール、パピュロキャンディン、ニューモキャンディン、エキノキャンディン、ポリオキシン、ニッコーマイシン、プラジミシン、ベナノミシンなどの抗真菌薬（"Antibiotics That Inhibit Fungal Cell Wall Development" Annu. Rev. Microbiol. 1994, 48: 471-97(その内容は参照により本明細書に組み入れる)を参照されたい）；抗不安薬、胃腸薬、中枢神経系活性化剤、鎮痛剤、排卵誘発剤または避妊薬、抗炎症薬、ステロイド系薬剤、抗尿酸血症薬、心血管作動剤、血管拡張薬、血管収縮薬などが挙げられる。
【００７１】
上記のものは本発明のプロドラッグに好適である生物学上活性な成分の例示である。特記していないが、好適なエステル形成基、すなわち、ヒドロキシル基を有する生物学上活性な材料もまた本発明の範囲とされるものと理解すべきである。また、本発明のプロドラッグ複合体が、1当量の薬物および高分子だけでなくin vivoにおいて生物活性に影響を及ぼさない成分を含有する少量の化合物も含有してよいことも理解すべきである。例えば、いくつかの例では、二酸と１個の結合ポイントを有する薬物分子とを反応させても、その反応条件では高分子当たり2当量の薬物を有するプロドラッグが定量的な量で生成されないということが分かっている。カルボジイミドを使用する場合にはアシル尿素などの反応副生成物が生じる場合がある。
【００７２】
2.アミン基含有化合物の残基

本発明のいくつかの態様では、B_1またはB_2はアミン基含有化合物の残基である。限定されるものではないが、かかる好適な化合物としては、有機化合物、酵素、タンパク質、ポリペプチドなどの残基が挙げられる。有機化合物としては、限定されるものではないが、ダウノルビシン、ドキソルビシン；p-アミノアニリンマスタード、メルファラン、Ara-C(シトシンアラビノシド)などをはじめとするアントラサイクリン系化合物、および関連代謝拮抗性化合物、例えば、ゲムシタビン、などの成分が挙げられる。あるいは、Bはアミン基含有心血管作動剤、抗新生物薬、抗感染症薬、ナイスタチンおよびアムホテリシンBなどの抗真菌薬、抗不安薬、胃腸薬、中枢神経系活性化剤、鎮痛薬、排卵誘発剤、避妊薬、抗炎症薬、ステロイド系薬剤、抗尿酸血症薬、血管拡張薬、血管収縮薬などの残基でありうる。

【００７３】

本発明の好ましい態様では、アミノ基含有化合物は、動物、例えば、ヒトをはじめとする哺乳類のかかる治療が望まれる症状の治療における医薬上のまたは診断上の使用に好適な、生物学上活性な化合物である。上記のものは例示を意図するものであり、改変しうる化合物を限定するものではない。当業者ならば、その他のかかる化合物も過度な試験を行うことなく同様に改変しうることが分かるであろう。特に示していないが、好適なアミノ基を有する生物学上活性な材料もまた本発明の範囲とされるものと理解すべきである。

【００７４】

本発明において含有させるのに好適なアミノ基含有分子のタイプについての唯一の条件は、担体部分と反応しかつそれと結合しうる利用可能な少なくとも1つの(第1または第2)アミン基含有位置が存在することと、プロドラッグ系が親化合物を放出して、親化合物を再生利用した後に生物活性の実質的な喪失がないことである。

【００７５】

本発明のプロドラッグ組成物への含有に好適な親化合物は、それ自体が結合型組成物からの加水分解による放出後は活性ではないが、さらなる化学工程/反応を受けた後に活性となりうる物質/化合物であってよいことに注目されたい。例えば、ダブルプロドラッグ輸送系によって血流に送達される抗癌剤は、癌または腫瘍細胞に浸透するまで不活性な状態にあり、そこで癌または腫瘍細胞化学、例えば、その細胞に特異的な酵素反応により活性化されると考えられる。

【００７６】

3.脱離基

B_1またはB_2が脱離基である態様では、好適な脱離基としては、限定されるものではないが、N-ヒドロキシベンゾトリアゾリル、ハロゲン、N-ヒドロキシフタルイミジル、p-ニトロフェノキシ、イミダゾリル、N-ヒドロキシスクシンイミジル；チアゾリジニルチオンなどの基を挙げることができ、あるいは当業者には理解されるその他の好適な脱離基が挙げられる。本明細書において使用し記載する合成反応は過度の試験を行わなくとも当業者ならば分かるであろう。

【００７７】

例えば、化合物(I)のアシル化中間体をクロロ蟻酸4-ニトロフェニル、ジスクシンイミジルカーボネート(DSC)、カルボニルジイミダゾール、チアゾリジンチオンなどの反応物質と反応させて所望の活性化誘導体を得ることができる。

【００７８】

p-ヒドロキシベンジルアルコールまたはp-アミノベンジルアルコール、およびo-ヒドロキシベンジルアルコールまたはo-アミノベンジルアルコールのフェノールまたはアニリン部分の選択的アシル化は、例えば、チアゾリジンチオン活性化高分子、スクシンイミジルカーボネート活性化高分子、カルボン酸活性化高分子、ブロックアミノ酸誘導体を用いて行うことができる。適切に実施されれば「活性化」型PEGプロドラッグ(またはブロックプロドラッグ)はアミンまたはヒドロキシル基含有化合物との複合体化が可能である。

【００７９】

F.高分子プロドラッグ輸送系の合成

典型的な高分子プロドラッグの合成を実施例で記載するが、一般に、プロドラッグ輸送系を製造する1つの好ましい方法では、最初に高分子残基を分枝基に結合させる。別途、生物学上活性な成分または薬物、例えば、薬物-OHまたは薬物-NH_2（式IのB_1またはB_2）をTML成分に結合させる。このTML成分は、高分子との結合ポイントに二官能性スペーサーを含んでもよい。次に、末端分枝を有する高分子残基と薬物-TML成分とを最終生成物を生成するのに十分な条件下で反応させる。

【００８０】

二官能性スペーサーを含有するTML-薬物成分と高分子部分との結合は、好ましくはカップリング剤の存在下で行われる。限定されるものではないが、好適なカップリング剤としては、例えば、Sigma-Aldrich Chemicalなどの民間供給会社から入手可能である、または公知の方法により合成される1,3-ジイソプロピルカルボジイミド(DIPC)、好適なジアルキルカルボジイミド、ハロゲン化2-ハロ-1-アルキル-ピリジニウム、(Mukaiyama試薬)、1-(3-ジメチルアミノプロピル)-3-エチルカルボジイミド(EDC)、プロパンホスホン酸環状無水物(PPACA)およびジクロロリン酸フェニルなどが挙げられる。

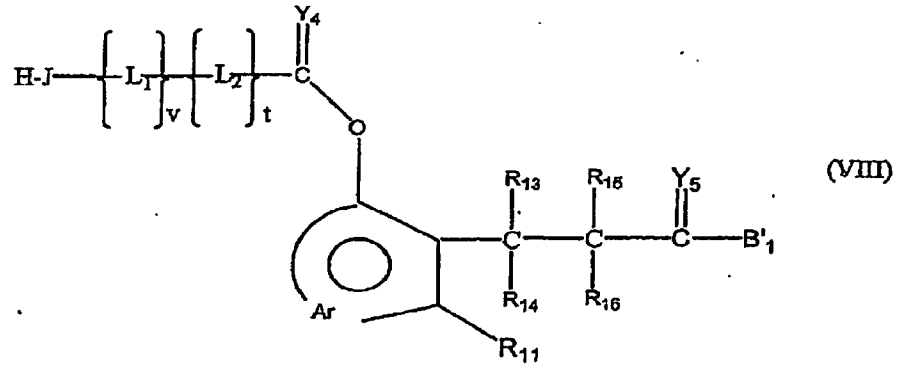
【００８１】

好ましくは、置換基を塩化メチレン、クロロホルム、DMFまたはその混合物などの不活性溶媒中で反応させる。この反応は、好ましくは生成した全ての酸を中和するためにジメチルアミノピリジン、ジイソプロピルエチルアミン、ピリジン、トリエチルアミンなどの塩基の存在下、0℃～約22℃(室温)の温度で行われる。

【００８２】

より詳細には、高分子輸送系を製造する1つの方法は式(VIII):

【化２５】

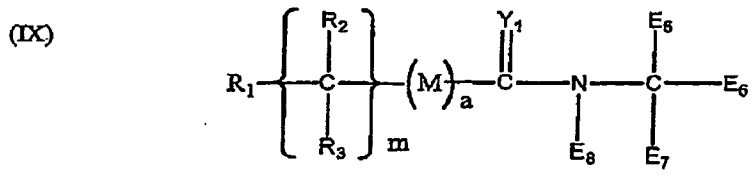


(VIII)

【００８３】

(式中、
全ての置換基および変数はこれまでに定義したとおりであり、かつ
B'_1はヒドロキシルまたはアミン基含有成分の残基である)
で表される化合物と式(IX):

【化２６】

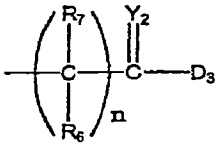


(IX)

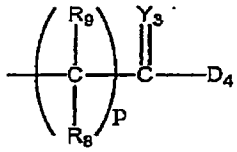
【００８４】

(式中、
R_1は高分子残基であり；

Y_1はO、SまたはNR_4であり，
Mは O、SまたはNR_5であり；
(a)は0または1であり；
(m)は0または正の整数であり；
Y_{2-3}は独立に、O、SまたはNR_{10}であり；かつ
R_{2-3}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
E_5は
【化２７】

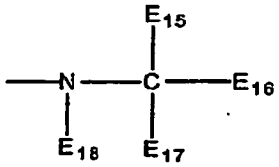


【００８５】
であり；
E_{6-8}は独立に、H、E_5または
【化２８】



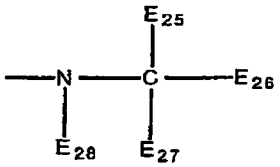
【００８６】
(式中、
D_3およびD_4は独立に、OHまたは保護されていないアミンまたはヒドロキシルと反応しうる脱離基、または末端分枝基であり；
(n)および(p)は独立に、0または正の整数であり；
Y_{2-3}は独立に、O、SまたはNR_{10}であり；かつ
R_{6-10}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択される）
である}
で表される化合物とを反応させることを含む。
【００８７】
この方法のさらなる態様では、D_3およびD_4は独立に、式(X)
【化２９】

(X)

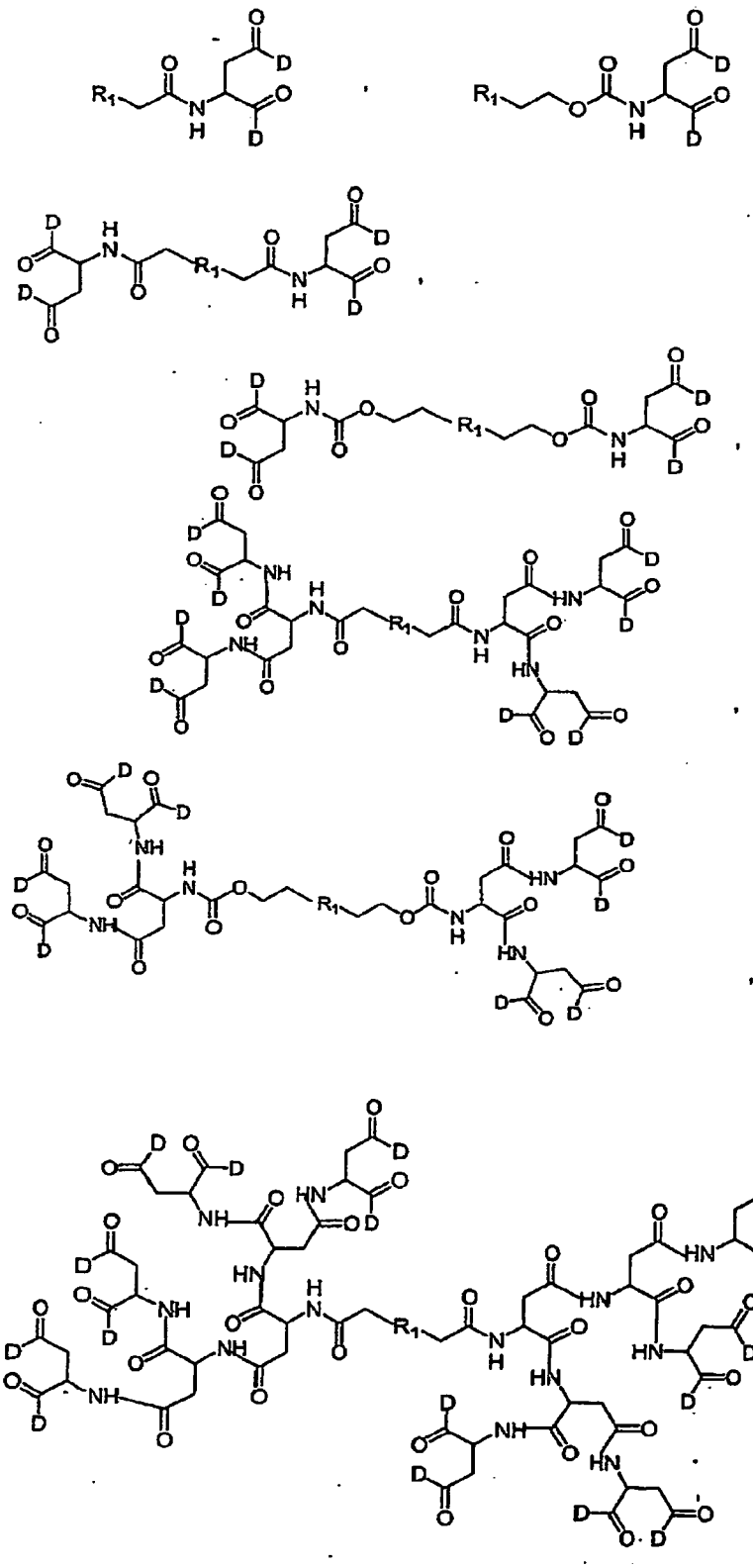


【００８８】
(式中、
E_{15-18}はD_3およびD_4が以下で定義するD'$_3$およびD'$_4$に変わることを除き、E_{5-8}の定義と同じ基から選択される)
で表される選択された末端分枝基である。この実施形態では、D'$_3$およびD'$_4$が独立に、OH、式(IV)または(V)で表される成分、または(XI)
【化３０】

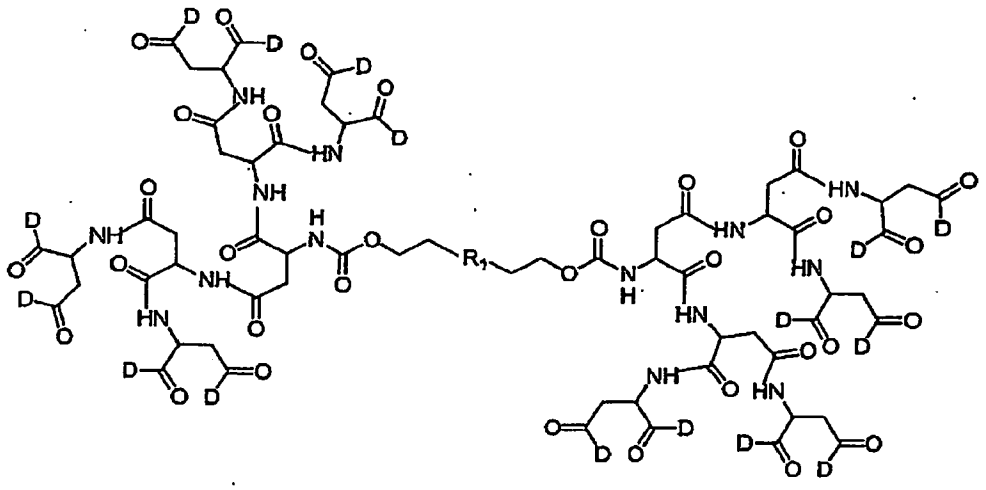
(XI)



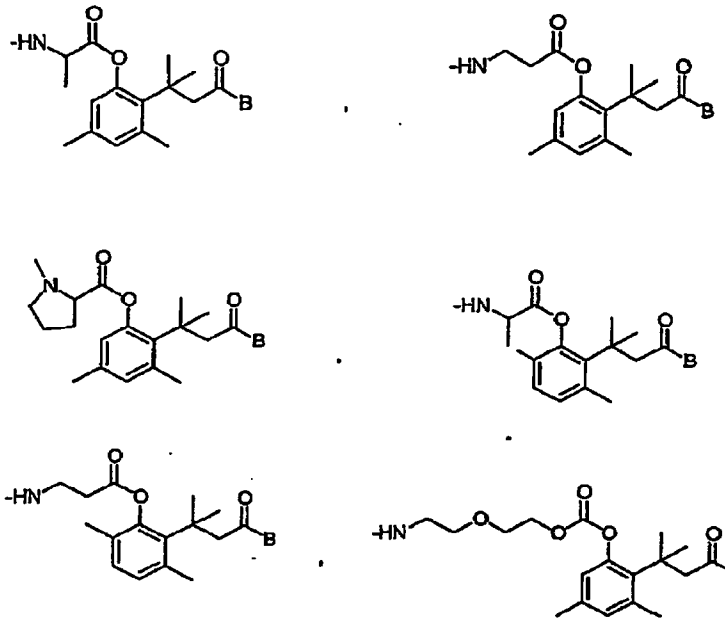
【００８９】
(式中、
E_{25-28}はD_3およびD_4がD"$_3$およびD"$_4$に変わり、かつD"$_3$およびD"$_4$が独立に、OH、または保護されていないアミンもしくはヒドロキシルと反応しうる脱離基と定義されることを除き、E_{5-8}の定義と同じ基から選択される)
で表される成分でありうる。
【００９０】
かかる合成方法により、最大16当量のカルボン酸または活性化カルボン酸を、例えば、結合させることが可能である。本明細書における好ましい構造で示されるように、末端分枝多酸を有するPEG残基が本発明の好ましい態様である。
【００９１】
選択された合成方法にかかわらず、本明細書において記載する合成方法によって得られる好ましい化合物としては、
【化３１】



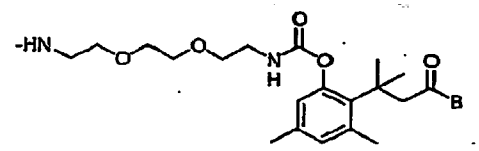
【００９２】
および
【化３２】



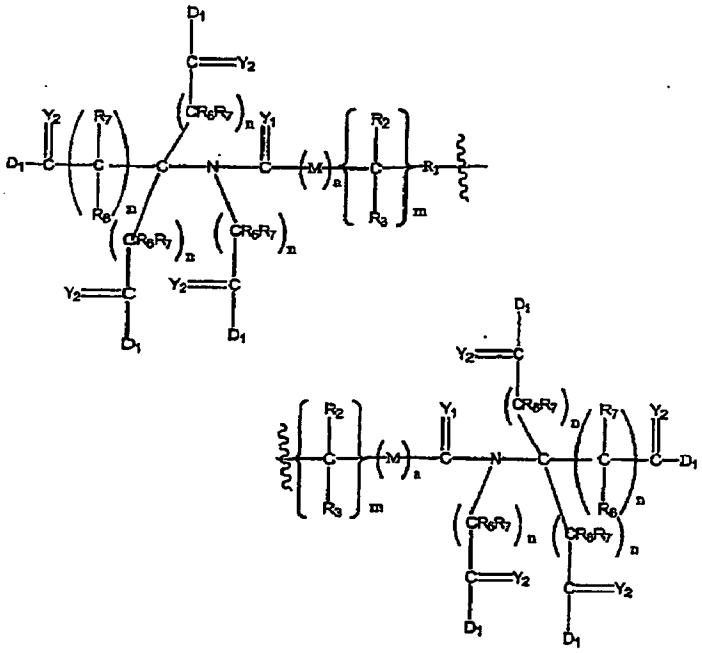
【００９３】
{式中、
R_1はPAOまたはPEGなどの高分子残基であり、かつDはOH、式(IV)または(V)である。好ましくは、Dは
【化３３】



【００９４】
または
【化３４】



【００９５】
(式中、
Bはアミンまたはヒドロキシル基含有薬物の残基である)
である}
が挙げられる。
【００９６】
本発明のもう1つの好ましい態様では、本発明の化合物は式(XII):
【化３５】



【００９７】
(式中、
全ての置換基および変数はこれまでに定義したとおりである)
で表される。
【００９８】
G.in vivo診断学
本発明のさらなる態様は、所望により、診断または造影目的に選択される診断タグを上記の輸送エンハンサーに付けて作製してもよい本発明の複合体を提供する。そのため、好適なタグは、好適な成分、例えば、アミノ酸残基を、当技術分野の標準放射性同位元素、放射線不透過性標識、磁気共鳴標識、またはその他、磁気共鳴映像法に好適なその他の非放射性同位元素標識、蛍光標識、外科処置中の腫瘍組織のイメージングを可能にする可視色を呈する標識および/または紫外線、赤外線または電気化学的刺激下で蛍光発光可能な標識などに結合させることにより作製される。所望により、診断タグを複合体化される治療成分に組み込みおよび/または結合させて、動物またはヒト患者内での治療用生物活性材料の分布のモニタリングを可能にすることができる。

【００９９】
本発明のなおさらなる態様では、本発明のタグ付き複合体が当技術分野で公知な方法により、例えば、放射性同位元素標識をはじめとする好適な標識を用いて容易に作製される。一例として、これらにはin vivoにおいて腫瘍細胞に選択的に取り込まれる放射免疫シンチグラフ用薬剤を製造するための^{131}ヨウ素、^{125}ヨウ素、^{99m}テクネチウムおよび/または^{111}インジウムが挙げられる。例えば、一例として、参照により本明細書に組み入れる米国特許第5,328,679号；同第5,888,474号；同第5,997,844号；および同第5,997,845号により示されたものをはじめとする、ペプチドをTc-99mに結合させる当技術分野で公知な方法が多数ある。

【０１００】
一般には、患者の腫瘍組織の解剖学的位置決定では、腫瘍を有することが予測される患者または動物に複合体タグを投与する。標識化免疫グロブリンを腫瘍部位に位置付けるのに十分な時間が経過した後、標識により発生するシグナルを、例えば、X線ラジオグラフィー、コンピュータ体軸横断X線断層撮影、MRIにより、発光性タグの機器検出により、ガンマカメラなどのフォトスキャン装置、または選択されたタグの性質に好適なその他の方法もしくは装置により視覚的に検出する。

【０１０１】
次いで、検出されたシグナルを画像または腫瘍部位の解剖学的および/もしくは生理学的判定に変換する。この画像によりin vivoにおける腫瘍の位置付けが可能になり、好適な治療計画の立案ができる。タグ付き成分自体が治療薬である実施形態では、検出されたシグナルによって治療中の解剖学的位置決定が明らかであり、診断的および治療的インターベンションを追跡するための基準が提供される。

【０１０２】
H.治療方法
本発明のもう1つの態様により、哺乳類における種々の病状に向けた治療方法が提供される。これらの方法は、かかる病状の治療が必要な哺乳類に、有効量の、本明細書において記載するように製造したマルチ・ローディッド(multi-loaded)Ara-C-PEG複合体などのプロドラッグを投与することを含む。該組成物は特に、新生物性疾患を治療する、全身腫瘍組織量を減少させる、新生物転移を予防する、および哺乳類における腫瘍/新生物増殖の再発を予防するのに有用である。

【０１０３】
投与するプロドラッグ量はその中に含まれる親分子に応じたものとなる。一般に、治療方法に使用するプロドラッグ量は哺乳類において所望の治療効果を効果的に達成する量である。必然的に、種々のプロドラッグ化合物の投与量は親化合物、in vivo加水分解速度、高分子の分子量などによっても多少変わるが、一般には、タキサン系プロドラッグはタキサン部分の量を基に1日当たり約5～約500mg/m^{2}の範囲の量で投与される。また、カンプトテシンプロドラッグも1日当たり約5～約500mg/m^{2}の範囲の量で投与される。上記の範囲設定は例示であり、臨床経験および治療適用に基づき、選択されたプロドラッグの最適投与量が当業者により決定されるであろう。実際の投与量については必要以上の試験を行うことなく、当業者には明らかであろう。

【０１０４】
哺乳類へ投与するために本発明のプロドラッグを1種以上の好適な医薬組成物に含めることができる。医薬組成物は当技術分野で十分に公知な方法に従って製造される液剤、懸濁剤、錠剤、カプセル剤などの形態であってよい。また、当業者が要すれば、かかる組成物の投与は経口および/または非経口経路によるものであってよいと考えられる。組成物の溶液および/または懸濁液は、例えば、当技術分野で公知な方法、例えば、静脈内、筋肉内、皮下注射などによる組成物の注入または浸潤用の担体ビヒクルとして使用しうる。

【０１０５】
また、かかる投与は体内スペースもしくは体腔への注入、ならびに吸入および/または経鼻経路によるものであってもよい。しかしながら、本発明の好ましい態様では、プロドラ

ッグはその必要のある哺乳類に非経口投与される。

【実施例】

【０１０６】

I.実施例

次の実施例は本発明をさらに理解するためのものであり、本発明の有効な範囲を何ら制限するものではない。実施例で列挙される下線を施した太字体の数字は図1～5で示されるものと対応している。

【０１０７】

概説

反応は全て乾燥窒素またはアルゴン雰囲気下で行った。市販の試薬はさらなる精製を行わずに使用した。全てのPEG化合物は使用前に真空下または共沸蒸留(トルエン)により乾燥させた。^{1}Hスペクトルは特に断りのない限り、溶媒としてジュウテリオクロロホルムを用いてJEOL FT NMR システム JNM GSX-270装置で測定した。^{13}C NMRスペクトルはJNM GSX-270では67.80MHzで測定した。化学シフト(σ)はテトラメチルシラン(TMS)から低磁場へ向かう百万分の一(ppm)単位で表され、結合定数(J値)はヘルツ(Hz)で示される。in vivo薬物処理前の注入用に全てのPEG複合体化化合物を滅菌生理食塩水(0.9%)に溶解し(～15mg/mL)、それらをara-C等価物として投与した(絶対量のara-Cを投与)。

【０１０８】

HPLC法

C8逆相カラム(Beckman, ultrasphere)を用い、移動相としてメタノール-水の80:20混合物(v/v)を用いる定組成条件下でHPLC分析を行った。ピーク溶出は紫外吸光検出器を用いて254nmでモニターした。遊離PEGの存在を検出し、さらにPEG化生成物の存在も確認するため、蒸発光散乱検出器(ELSD)、PL-EMD 950型(Polymer Laboratories)を使用した。ELSDおよびUV分析では、全ての最終PEG化生成物には非改変薬物が含まれず、HPLCによる純度は≧95%であった。

【０１０９】

PEG誘導体中のAra-C含量の分析

PEG誘導体中のara-C含量を測定するため、N^4-アセチルシチジンを標準として使用した。H_2O中のN^4-アセチルシチジンのUV吸光度を0.01μmol/mL～0.05μmol/mLの範囲にわたる6種の異なる濃度で257nmにおいて測定した。濃度に対する吸光度の検量線でのN^4-アセチルシチジンの吸光係数εの計算値は36.4であった(1mg/mLの257nmにおけるO.D.、光路長1.0cm)。PEG化ara-C誘導体をおよその濃度0.015μmol/mL(分子量40kDaを基にして)でH_2Oに溶解し、これら化合物のUV吸光度を257nmで測定した。この値と、さらに上記で得た吸光係数εを用いて、サンプル中のara-C濃度を調べた。この値をサンプル濃度で割ってサンプル中のara-Cの割合(%)を求めた。

【０１１０】

PEG誘導体中のメルファラン含量の分析

PEG誘導体中のメルファラン含量を測定するため、メルファランを標準として使用した。DMF-H_2O(9:1, v/v)中のメルファランのUV吸光度を0.02μmol/mL～0.06μmol/mLの範囲にわたる5種の異なる濃度で264nmにおいて測定した。濃度に対する吸光度の検量線でのメルファランの吸光係数εの計算値は54.6であった(1mg/mLの264nmにおけるO.D.、光路長1.0cm)。PEG化メルファラン誘導体をおよその濃度0.013μmol/mL(分子量40kDaを基にして)でDMF-H_2O (9: 1, v/v)に溶解し、これら化合物のUV吸光度を264nmで測定した。この値と、さらに上記で得た吸光係数εを用いて、サンプル中のメルファラン濃度を調べた。この値をサンプル濃度で割ってサンプル中のメルファランの割合(%)を求めた。

【０１１１】

略語

DCM(ジクロロメタン)、DMAP(4-(ジメチルアミノ)ピリジン)、EDC(1-エチル-3-(3-ジメチルアミノプロピル)カルボジイミド)、HOBT(1-ヒドロキシベンゾトリアゾール)、IPA(2-プロパノール)、NMM(N-メチルモルホリン)、TFA(トリフルオロ酢酸)。

【０１１２】
[実施例1] 化合物3a
無水ピリジン(50mL)中のara-C(1, 1.73g, 7.12mmol)、2a(700mg, 1.78mmol)、HOBT(0.96g, 7.12mmol)、およびEDC・HCl(2.73g, 14.25mmol)の混合物を室温で2時間撹拌し、温度を40℃に上昇させてこの反応を一晩続けた。溶媒を除去し、塩化メチレン(50mL)を用いて混合物を溶解した後、水(3×30mL)、次ぎに、0.1N HCl(2×30mL)で洗浄した。有機層を無水$MgSO_4$で乾燥させ、溶媒を真空除去すると粗生成物が得られた。これをシリカゲルカラムクロマトグラフィー(DCM中5～10%MeOH)により精製し、638.8mg(52%)の3aを白色の固体として得た:^{1}H NMRδ 1.42, 1.55, 2.17, 2.26, 2.46, 2.79, 3.84, 3.91, 4.14, 4.33, 4.53, 5.49, 6.07, 6.17, 6.52, 6.76, 7.31, 7.67, 8.16, 8.62;^{13}C NMR δ 17.77, 20.11, 25.36, 28.32, 31.51, 31.96, 39.57, 50.18, 50.45, 61.88, 74.50, 80.15, 85.90, 88.58, 96.25, 122.51, 132.82, 133.34, 136.73, 138.22, 146.57, 149.90, 155.65, 155.96, 162.08, 171.89, 174.06。

【０１１３】
[実施例2] 化合物3b
化合物1を実施例1と同様の条件を用いて2bと結合させ、3bを54%の収率で得た:^{13}C NMR δ 17.23, 17.92, 18.33, 25.49, 28.32, 31.51, 31.58, 31.99, 32.46, 39.52, 40.09, 50.08, 50.22, 61.72, 74.50, 74.94, 80.11, 80.15, 85.45, 85.90, 88.01, 88.58, 96.25, 122.51, 128.77, 129.03, 129.16, 131.68, 132.82, 136.24, 136.73, 138.22, 146.05, 146.57, 149.90, 155.65, 155.96, 171.85, 171.89, 174.06。

【０１１４】
[実施例3] 化合物4a
無水DCM(6mL)およびTFA(4mL)中、化合物3a(638.8mg, 1.03mmol)を室温で2時間撹拌した。この溶液にエチルエーテルを加えると粗生成物が沈殿した。これを濾過し、エーテルで洗浄し、4aを白色の固体として得た(534.5mg, 82%):^{1}H NMR (DMSO-d_6) δ 1.52 (s, 3H, (CH_3)$_2$CH) 1.55 (s, 3H, (CH_3)$_2$CH), 1.62 (d, 1 H, J= 8.1 Hz, (CH_3)$_2$CH), 2.22 (s, 3H, CH_3Ar), 2.57 (s, 3H, CH_3Ar), 2.97 (s, 2H, CH_2C(=O)), 3.41-4.27 (m, 5 H, ara-C's H-2'-H5'), 6.09 (d, 1H, J = 5.4, ara-C's H-1'), 6.67 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.12 (d, J= 5.4, H-6), 8.05 (d, J= 8.1, H-5), 8.67 (bs, 1H, TFA);^{13}C NMR (DMSO-d_6) δ 15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53, 49.02, 61.02, 64.94, 74.64, 76.14, 85.74, 86.95, 94.32, 122.32, 132.41, 134.08, 135.67, 138.09, 146.71, 149.20, 154.50, 158.21, 158.72, 162.02, 169.68, 171.87。

【０１１５】
[実施例4] 化合物4b
化合物3bを実施例3と同じ条件に付し、4bを82%の収率で得た:^{1}H NMR (DMSO-d_6) δ 1.52 (s, 3H, (CH_3)$_2$CH) 1.55 (s, 3H, (CH_3)$_2$CH), 1.62 (d, 1 H, J= 8.1 Hz, (CH_3)$_2$CH), 2.22 (s, 3H, CH_3Ar), 2.57 (s, 3H, CH_3Ar), 2.97 (s, 2H, CH_2C(=O)), 3.41-4.27 (m, 5 H, ara-C's H-2'-H5'), 6.09 (d, 1H, J = 5.4, ara-C's H-1'), 6.67 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.12 (d, J= 5.4, H-6), 8.05 (d, J = 8.1, H-5), 8.67 (bs, 1H, TFA);^{13}C NMR (DMSO-d_6) δ 15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53, 49.02, 61.02, 64.94, 74.64, 76.14, 85.74, 86.95, 94.32, 122.32, 132.41, 134.08, 135.67, 138.09, 146.71, 149.20, 154.50, 158.21, 158.72, 162.02, 169.68, 171.87。

【０１１６】
[実施例5] 化合物6a
無水DCM(50mL)中のPEG-アスパラギン酸(分子量40,000, 5, 3 g, 0.074mmol)、4a(385.6mg, 0.74mmol)、NMM(240mg, 2.38mmol)、HOBT(120.5mg, 0.89mmol)、およびEDC・HCl(228.4mg, 1.19mmol)の混合物を0℃で30分間撹拌した。この反応物を室温に温め、反応を3日間続け、濾過した。濾液を真空濃縮し、残渣をIPAで再結晶化させ、2.7g(90%)の生成物を得た。UVアッセイで測定した生成物中のara-C量は2.11重量%であった:^{13}C NMR δ 14.40, 1

9.22, 24.86, 31.17, 38.26, 38.90, 47.94, 48.67, 49.66, 60.17, 61.12, 61.90, 67.86-70.87 (PEG), 71.70, 74.50, 85.01, 87.53, 95.28, 121.39, 131.18, 132.68, 133.19, 134.77, 137.70, 145.26, 138.93, 155.23, 160.12, 161.56, 168.39, 170.72, 170.92, 171.27, 171.34。

【0117】

[実施例6] 化合物6b

化合物4bを実施例5と同じ条件に付し、6bを88%の収率で得た。UVアッセイで測定した生成物中のara-C量は1.68重量%であった:^{13}C NMRδ 15.12, 16.22, 24.52, 24.73, 29.55, 30.55, 31.15, 38.04, 38.59, 47.66, 49.16, 49.93, 50.18, 60.93, 61.12, 62.90, 69.44-71.59 (PEG), 71.70, 74.50, 84.78, 84.90, 87.53, 94.85, 127.60, 130.20, 135.51, 136.10, 141.70, 145.15, 147.50, 155.00, 161.20, 169.47, 170.62, 170.92, 171.27。

【0118】

[実施例7] 化合物9

PEGジオール(7, 55g, 1.38mmol)を2時間トルエンと共沸させた後、回転蒸発により200mLの溶媒を除去した。この溶液を～30℃に冷却し、トリホスゲン(0.544g, 1.83mmol)を固体として、次ぎに、無水ピリジン(0.434g, 5.49mmol)を加え、反応混合物を50℃で1時間撹拌した。N-ヒドロキシフタルイミド(8, 1.12g, 6.88mmol)および無水ピリジン(0.54g, 6.88mmol)をクロロ蟻酸混合物に加え、反応物を50℃でさらに2時間、次ぎに、室温で12時間撹拌した。この反応混合物を濾紙で濾過し、溶媒を真空除去し、生成物を塩化メチレン-エチルエーテル(1100mL, 8:2, v/v)で再結晶化させ、生成物を得た(50.9g, 92%):^{13}C NMR δ 123.62, 128.10, 134.55, 152.00, 160.00。

【0119】

[実施例8] PEG-cmc-Asp-O-t-Bu(11)

化合物9(分子量40,000, 20g, 0.459mmol)およびアスパラギン酸ジt-ブチルエステルHCl(10, 1.0g, 3.55mmol)を無水DCMに溶解した後、DMAP(0.433g, 3.55mmol)を加えた。この溶液を一晩還流した後、エチルエーテル(1L)を加えて沈殿させた。濾過により固体を単離し、IPA(1L)で2回再結晶化させた。濾過ケーキをIPA(200mL)およびエーテル(200mL)で洗浄し、45℃で真空乾燥させた後に15.6g(78%)の生成物を得た:^{13}C NMRδ 27.837 ($CH_2CO_2C(CH_3)_3$), 27.991 ($CHCO_2C(CH_3)_3$), 37.752 ($CHCH_2CO_2$), 50.800 (NHCH), 64.212 ($OCH_2CH_2OC(=O)NH$), 81.333 ($CH_2CO_2C(CH_3)_3$), 82.007 ($CHCO_2C(CH_3)_3$), 155.924 ($OCH_2CH_2OC(=O)NH$), 169.674 ($CH_2CO_2C(CH_3)_3$), 169.969 ($CHCO_2C(CH_3)_3$)。

【0120】

[実施例9] PEG-cmc-Asp-OH(12)

化合物11(15g, 0.375mmol)をDCM(150mL)に溶解した後、TFA(75mL)を加えた。この溶液を室温で2時間撹拌し、ヘキサン(500mL)を加えて固体を沈殿させた。この固体をヘキサンでトリチュレートしてTFAを除去した後、冷却したDCM-エーテルで再結晶化させた。再結晶化させた固体をDCM(150mL)に再溶解し、水(150mL)で洗浄した。有機層を分離し、無水$MgSO_4$で乾燥させ、真空濃縮し、エーテルで沈殿させ、12.4g(83%)の生成物を得た:^{13}C NMRδ 36.441 ($CHCH_2CO_2$), 50.177 (NHCH), 64.390 ($OCH_2CH_2OC(=O)NH$), 81.333 ($CH_2CO_2C(CH_3)_3$), 82.007 ($CHCO_2C(CH_3)_3$), 156.172 ($OCH_2CH_2OC(=O)NH$), 171.944 ($CH_2CO_2C(CH_3)_3$), 172.211 ($CHCO_2C(CH_3)_3$)。

【0121】

[実施例10] Boc-Asp-Asp-OMe(15)

EDC・HCl(2.47g, 12.86mmol)を無水DCM(30mL)およびDMF(2mL)中のBocNH-アスパラギン酸(13, 1g, 4.29mmol)、アスパラギン酸ジメチルエステル・HCl(14, 1.86g, 9.43mmol)、およびDMAP(2.47g, 12.86mmol)の混合物に0℃で加えた。この混合物を室温に一晩温めた。混合物を1N HClで3回洗浄し、有機層を無水$MgSO_4$で乾燥させた後、溶媒を真空除去し、生成物を得た(2.0g, 90%):^{1}H NMRδ 1.45 (s, 9H), 2.62-3.02 (m, 6H, 3 x CH), 3.70 (s, 6H, 2 x OCH_3), 3.74 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.50 (bs, 1H, CH), 4.85 (m, 2H, 2 x CH), 6.05 (d, J = 6.95 Hz, 1H, NH), 6.98 (d, J = 8.05 Hz, 1H, NH), 7

.57 (d, J = 7.69 Hz, 1H, NH)。

【0122】

[実施例11] Asp-Asp-OMe(16)

化合物15(2.0g, 3.85mmol)をDCM(30mL)およびTFA(15mL)に溶解し、溶液を室温で2時間撹拌した。溶媒を真空除去し、残渣をDCM-エーテルで2回再結晶化させ、生成物(1.74g, 87%)を白色の固体として得た:^{13}C NMR δ 35.52, 48.76, 50.12, 51.90, 51.96, 52.65, 114.59, 118.49, 168.43, 170.02, 170.92, 171.17, 171.40, 171.48。

【0123】

[実施例12] PEG-cmc-Asp-Asp-OMe(17)

DMAP(4.5g, 36.86mmol)を700mLの無水クロロホルム中の9(分子量40,000, 74g, 1.84mmol)および16(9.83g, 18.43mmol)の溶液に加えた。この反応混合物を窒素下、24時間還流した。反応物を室温まで冷却し、濃縮して1/4の量にした。粗生成物を2.5Lのエーテルで沈殿させ、濾過し、5.5LのIPA(65℃)で再結晶化させた。生成物を濾過し、新鮮なIPAで2回、新鮮なエーテルで2回洗浄し、40℃で一晩乾燥させ、59.0g(84%)の17を得た:^{13}C NMR δ 35.344, 36.931, 48.082, 48.208, 50.835, 51.509, 52.239, 61.045, 63.953, 68.854-72.056, 155.538, 170.102, 170.369, 170.453, 170.734。

【0124】

[実施例13] PEG-cmc-Asp-Asp-OH(18)

化合物17 (51g, 1.26mmol)およびLiOH・H_2O(0.8g, 18.9mmol)を300mLの水に溶解し、溶液を室温で一晩撹拌した。1N HClを加えて溶液のpHを2.5に調整した。溶液をDCM(3×600mL)で抽出し、有機層を合し、無水$MgSO_4$で乾燥させ、真空濃縮した。残渣をDCMエーテルで再結晶化させると生成物が得られた。これを濾過により回収し、40℃で一晩乾燥させ、38g(54%)のオクタ酸(octa-acid)を得た:^{13}C NMR (D_2O) δ 38.384, 39.704, 51.951, 54.465, 62.934, 67.105, 71.445-74.381 (PEG), 159.772, 173.831, 174.940, 176.359, 176.696。

【0125】

[実施例14] Mel-OMe(20)

メルファラン(19, 1.00g, 3.28mmol)を2,2-ジメトキシプロパン(65.59mL, 533.49mmol)に懸濁した。この懸濁液にHCl水溶液(36%, 3.28mL)および無水メタノール(4mL)を加えた。混合物を、溶液がわずかに褐変し始めるまで激しく撹拌しながら穏やかに加温還流した後、室温で18時間撹拌した。反応混合物を真空濃縮し、残渣から粗生成物をエーテルで沈殿させた。固体を濾過し、エーテルで洗浄し、シリカゲルカラムクロマトグラフィー($CHCl_3$:MeOH = 9:1, v/v)により精製し、所望の生成物を得た(0.47g, 45%):^{13}C NMR δ 39.751, 40.340, 51.912, 53.435, 55.803, 112.124, 126.076, 130.620, 145.033, 175.754。

【0126】

[実施例15] Boc-TML1β-Mel-OMe(22)

氷浴中0℃でEDC(0.52g, 2.70mmol)およびDMAP(0.988g, 8.10mmol)を無水DCM(15mL)および無水DMF(5mL)中の21(0.531g, 1.35mmol)および20(0.863g, 2.70mmol)の混合物に加えた。窒素下、反応混合物を室温で一晩撹拌した後、真空濃縮した。残渣をDCM(75mL)に再溶解し、25mL 1N HClで3回洗浄した。有機層を無水硫酸マグネシウムで乾燥させ、濃縮し、シリカゲルカラムクロマトグラフィー(酢酸エチル:ヘキサン = 7:3, v/v)により精製し、所望の生成物を得た(0.757g, 80.8%):^{13}C NMR δ 20.120, 25.306, 28.294, 31.768, 35.427, 35.947, 36.669, 39.505, 40.311, 49.324, 51.959, 53.234, 53.453, 79.467, 112.095, 123.374, 125.169, 130.439, 132.856, 133.427, 136.666, 138.697, 145.091, 149.841, 156.081, 170.888, 172.298。

【0127】

[実施例16] TML1β-Mel-OMe TFA塩(23)

化合物22(0.757g, 1.09mmol)をDCM(5mL)およびTFA(2.5mL)中、室温で2時間撹拌した。この反応液を濃縮し、最少量のDCMに再溶解し、エーテルで沈殿させた。生成物を濾過により回収し、所望の生成物を得た(0.222g, 35.9%):^{13}C NMR ($CDCl_3$ + CD_3OD) δ 20.026, 25

.146, 31.738, 31.892, 35.271, 36.219, 39.163, 40.340, 49.006, 52.219, 53.396, 112.073, 123.260, 124.756, 130.377, 133.026, 133.180, 136.815, 138.595, 145.110, 149.283, 171.069, 171.619, 172.630。

【0128】

[実施例17] PEG-cmc-TML1β-Mel-OMe(24)

窒素下、無水DCM(23mL)および無水DMF(6mL)中のPEG-cmc-Asp-Asp-OH(12, 1.6g, 0.0391mmol)、23(0.277g, 0.391mmol)、EDC(0.076g, 0.391mmol)、およびDMAP(0.155g, 1.269mmol)の混合物を室温で一晩撹拌した。この溶液を真空濃縮し、残渣を130mL IPAで再結晶化させ、生成物を得た(1.543g, 92.5%)。UVアッセイで測定した生成物中のメルファラン量は2.86重量%であった:^{13}C NMR δ 19.642, 24.788, 31.175, 34.350, 35.975, 38.817, 39.905, 48.558, 51.553, 52.808, 60.897, 62.331, 65.145-72.878 (PEG), 111.394, 122.761, 124.425, 129.698, 132.105, 132.878, 135.804, 137.737, 144.316, 149.065, 160.432, 170.608, 171.598。

【0129】

[実施例18] Boc-TML1β-AraC(25)

無水ピリジン(85mL)中のAra-C(1, 9.88g, 40.66mmol)の溶液を無水ピリジン(200mL)中の21(4.0g, 10.17mmol)、HOBT(5.49g, 40.66mmol)、EDC(15.61g, 81.32mmol)、およびNMM(8.93mL, 8.21g, 81.32mmol, 8当量)の混合物に加えた。窒素下、この反応混合物を40℃で48時間撹拌した後、真空濃縮した。残渣をDCM(300mL)に再溶解し、水(100mL)で3回、0.1N HCl(100mL)で2回洗浄した。有機層を硫酸マグネシウムで乾燥させ、濃縮し、シリカゲルカラムクロマトグラフィー($CHCl_3$:MeOH = 9:1, v/v)により精製し、所望の生成物を得た(3.26g, 52%):^{13}C NMR δ 20.315, 25.560, 28.522, 31.660, 35.520, 36.200, 39.221, 50.239, 61.719, 75.171, 76.698, 79.635, 85.341, 88.052, 96.435, 122.894, 132.519, 133.190, 136.186, 138.007, 146.222, 149.109, 155.906, 162.191, 171.733。

【0130】

[実施例19] TML1β-AraC TFA塩(26)

化合物25(3g, 4.85mmol)をDCM(15mL)に溶解した後、0℃でTFA(7.5mL)を加えた。この反応混合物を0℃で1.2時間撹拌し、冷水浴中で真空濃縮した。残渣をDCM-エーテルで沈殿させ、所望の生成物を得た(2.37g, 77%):^{13}C NMR ($CDCl_3$ + CD_3OD) δ 20.0, 25.3, 31.5, 31.7, 35.0, 38.9, 50.2, 60.9, 75.1, 75.8, 85.7, 88.1, 94.9, 109.7, 113.5, 117.3, 121.1, 122.5, 132.6, 136.4, 138.4, 148.7, 149.5, 150.1, 159.2, 159.6, 160.1, 160.6, 161.1, 170.6, 172.7。

【0131】

[実施例20] PEG-cmc-Asp-Asp-TML1β-AraC、八量体(27)

化合物26および18を実施例18と同じ条件に付し、27を製造した。

【0132】

[実施例21] 化合物6aおよび6bのin vitroおよびin vivoデータ

この実施例では、in vivoおよびin vitroデータを示し、非改変Ara-Cと比較している。

【0133】

in vivo

胸腺欠損ヌードマウスにドナーマウスから採取したLX-1の4～5mm^{3}組織片を皮下移植した。腫瘍トロカール部位を週2回観察し、触診できるものを週1回測定した。各マウスの腫瘍体積を測径器での二次元測定により調べ、式: 腫瘍体積 = (長さ×幅^2)/2により算出した。腫瘍が平均体積90mm^{3}に達したときに、マウスを非改変Ara-CおよびPEG-Ara-C化合物からなるそれらの試験群に分けた。腫瘍サイズ分布が均等になるようマウスを分類し、4～6マウス/群に群分けし、永久識別のため耳にパンチ穴を開けた。薬物を毎分約0.5mLの速度で尾部静脈から静脈投与した、q3d×4(1、4、7および10日目)。化合物は、20mg/kgの等モルベース(絶対量の活性成分)で、またそれら各々のMTD(Ara-C、100mg/kg/投与量(毒性); 6aおよび6b, 40mg/kg/投与量(容量))に近い値でも与えた。マウス重量および腫瘍サイズは試験開始時と、第4週まで週2回測定した。薬物の有効性は処置したマウスと処置してい

ない対照マウス(ビヒクルなし)との腫瘍増殖の比較により判定した。比較基準として5種類の指標を用いた：(a)28日目における平均腫瘍体積；(b)各腫瘍体積の試験開始時からの平均変化率；(c)対照群の腫瘍体積中央値が約800～1100mm³に達したとき(対数増殖期)に測定した腫瘍体積の相対率(%T/C)；(d)21日目における(～2000mm³)腫瘍体積の相対率(%T/C)および(e)各群の腫瘍寛解(28日目の腫瘍体積が1日目と比べて小さくなっている)数。

【０１３４】

結果

化合物6bはわずか20%の活性親化合物量で非改変Ara-Cよりも優れた抗腫瘍活性を示した。また、化合物6aも有意な効果を示した。%T/Cは6bの値の約2倍であったが、それにもかかわらず、特に本発明の化合物がわずか20%の活性親化合物量で投与されたことを考慮すれば、それは非改変Ara-Cと比較して都合がよい。

【表１】

化合物	$t_{1/2}$ (h)[a] ラット血漿	IC_{50} (nM)[a] P388/0	LX-I %T/C[b]
Ara-C	-	10	74.0 (100mg/kg)
化合物 6a	2.1	123	122 (20mg/kg)
化合物 6b	53	958	59.3 (20mg/kg)

【０１３５】

[a]全ての試験は2連、37℃で行い、$t_{1/2}$はPEG誘導体の消失により測定した。測定値の標準偏差 = ±10%。

[b]平均ベースライン腫瘍体積は1000mm³であった。

【０１３６】

in vitroバイオアッセイ

P388/O(マウスリンパ系腫瘍，Southern Research Institute)細胞系を使用して一連のin vitroバイオアッセイを行い、非改変Ara-Cおよび化合物10のIC_{50}を調べた。P388/O細胞をRPMI 1640培地(Whittaker Bioproducts, Walkersville, Maryland)+10%FBS(Hyclone Inc., Logan UT)で培養した。バイオアッセイは抗生物質およびファンギゾンを含有するそれら各々の培地で行った。

【０１３７】

Ara-CをDMSOに溶解し、培地で好適な濃度に希釈した。PEG-Ara-C化合物を水に溶解し、培地で好適な濃度に希釈した。

【０１３８】

アッセイを96ウェルマイクロタイター細胞培養プレートで2連で行った。

【０１３９】

化合物の2倍連続希釈をマイクロタイタープレートで行った。0.1%トリプシン/ベルセンを加えて37℃でインキュベートすることにより細胞を分離した。10%FBSを含有する各細胞系に好適な培地を加えてトリプシンを不活性化した。マイクロタイタープレートの各ウェルに10,000個の細胞を加えた。3日後、代謝性標識色素Alamar Blueを製造業者のプロトコールに従って添加し、細胞増殖を測定した。試験化合物および参照化合物のIC_{50}値は上記に表で示している。

【０１４０】

本発明の好ましい実施形態であると現在考えられるものを記載してきたが、当業者ならば本発明の精神を逸脱しない限り、変形および改変をなしうることが分かるであろう。本発明の真の範囲にあるかかる変形および改変の全てを請求することを意図するものである。

【図面の簡単な説明】

【０１４１】

【図１】図１は、実施例１～６で記載する本発明の化合物を製造する方法の概略図である。

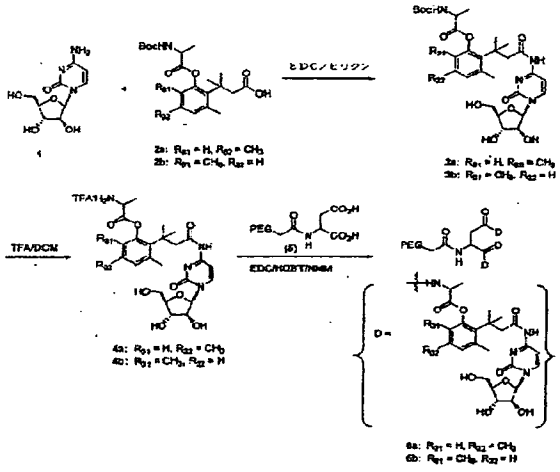
【図２】図２は、実施例７～９で記載する本発明の化合物を製造する方法の概略図である。

【図３】図３は、実施例１０～１３で記載する本発明の化合物を製造する方法の概略図である。

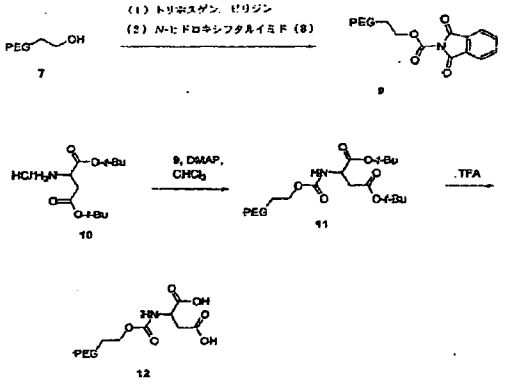
【図４】図４は、実施例１４～１７で記載する本発明の化合物を製造する方法の概略図である。

【図５】図５は、実施例１８～２０で記載する本発明の化合物を製造する方法の概略図である。

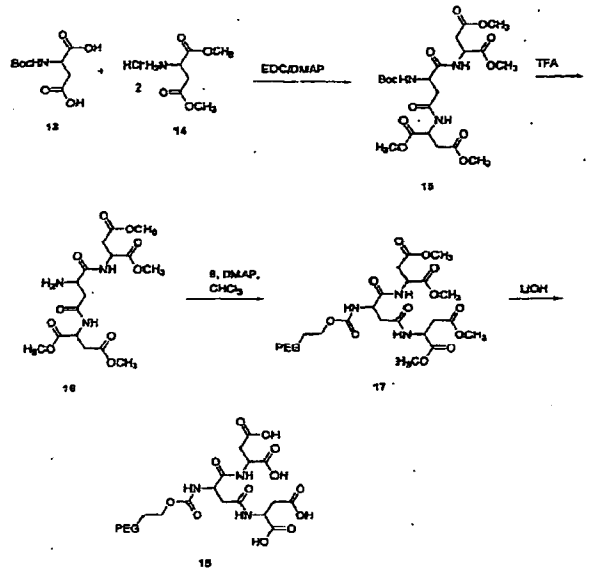
【図１】



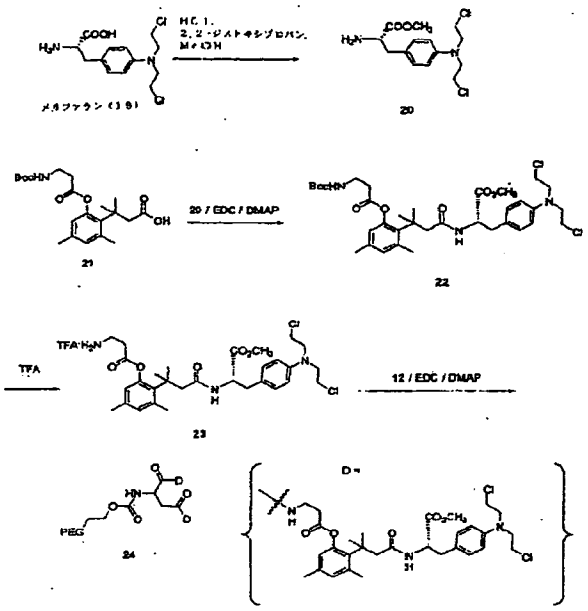
【図２】



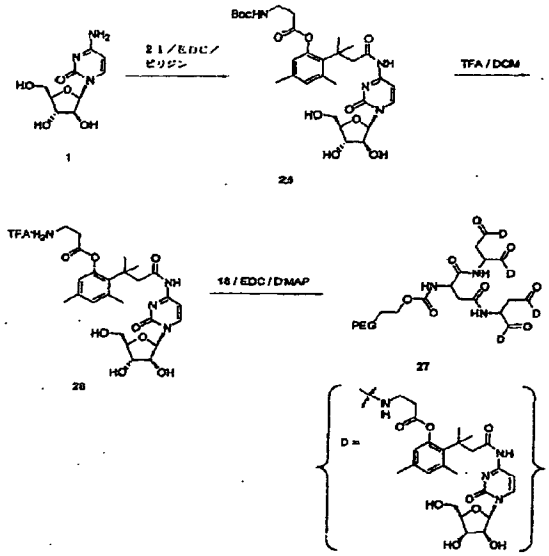
【図３】



【図４】



【図５】



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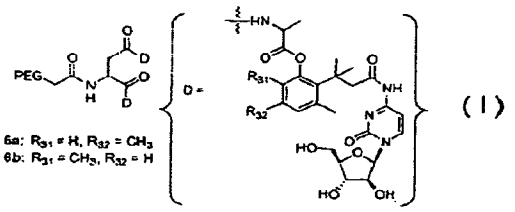
(54) Title: TERMINALLY-BRANCHED POLYMERIC LINKERS AND POLYMERIC CONJUGATES CONTAINING THE SAME



(I)

(57) Abstract: Terminally-branched polymeric prodrug platforms capable of high degrees of loading are disclosed. In preferred aspects of the invention, the prodrug platform releases multiple parent compounds after each branch holding the active agent undergoes a bond elimination reaction. Methods of preparing the prodrugs and using the same in the treatment of mammals are also disclosed. In one preferred aspect, polymeric conjugates such as formula (I) are provided.

WO 02/066066 A1

TERMINALLY-BRANCHED POLYMERIC LINKERS AND POLYMERIC CONJUGATES CONTAINING THE SAME

TECHNICAL FIELD

The present invention relates to new types of terminally-activated polymeric materials which are useful in forming long-acting conjugates of bioactive materials. In particular, the invention relates to polymeric-based conjugates having increased therapeutic payloads and methods of preparing the same.

BACKGROUND OF THE INVENTION

Over the years, several methods of administering biologically-effective materials to mammals have been proposed. Many medicinal agents are available as water-soluble salts and can be included in pharmaceutical formulations relatively easily. Problems arise when the desired medicinal agent is either insoluble in aqueous fluids or is rapidly degraded in vivo. Alkaloids are often especially difficult to solubilize.

One way to solubilize medicinal agents is to include them as part of a soluble prodrug. Prodrugs include chemical derivatives of a biologically-active parent compound which, upon administration, eventually liberate the parent compound in vivo. Prodrugs allow the artisan to modify the onset and/or duration of action of an agent in vivo and can modify the transportation, distribution or solubility of a drug in the body. Furthermore, prodrug formulations often reduce the toxicity and/or otherwise overcome difficulties encountered when administering pharmaceutical preparations. Typical examples of prodrugs include organic phosphates or esters of alcohols or thioalcohols. See Remington's Pharmaceutical Sciences, 16th Ed., A. Osol, Ed. (1980), the disclosure of which is incorporated by reference herein.

Prodrugs are often biologically inert or substantially inactive forms of the parent or active compound. The rate of release of the active drug, i.e. the rate of hydrolysis, is

influenced by several factors but especially by the type of bond joining the parent drug to the modifier. Care must be taken to avoid preparing prodrugs which are eliminated through the kidney or reticular endothelial system, etc. before a sufficient amount of hydrolysis of the parent compound occurs.

Incorporating a polymer as part of a prodrug system has been suggested to increase the circulating life of a drug. However, it has been determined that when only one or two polymers of less than about 10,000 daltons each are conjugated to certain biologically active substances such as alkaloid compounds, the resulting conjugates are rapidly eliminated in vivo, especially if a somewhat hydrolysis-resistant linkage is used. In fact, such conjugates are so rapidly cleared from the body that even if a hydrolysis-prone ester linkage is used, not enough of the parent molecule is regenerated in vivo to be therapeutic.

Camptothecin and related biologically active analogs are often poorly water soluble and are examples of substances which would benefit from PEG prodrug technology. A brief overview of some previous work in the field is presented below.

Ohya, et al., J. Bioactive and Compatible Polymers Vol. 10 Jan., 1995, 51-66, disclose doxorubicin-PEG conjugates which are prepared by linking the two substituents via various linkages including esters. The molecular weight of the PEG used, however, is only about 5,000 at most. Thus, the in vivo benefits are not fully realized because the conjugates are substantially excreted prior to sufficient linkage hydrolysis.

U.S. Patent No. 4,943,579 discloses certain simple 20(S)-camptothecin amino acid esters in their salt forms as water soluble prodrugs. The reference does not, however, disclose using an amino acid as part of a linkage which would attach the alkaloid to a relatively high molecular weight polymer in order to form a prodrug. As evidenced by the data provided in Table 2 of the '579 patent, hydrolysis is rapid. Consequently, at physiologic pH, the insoluble base is rapidly generated after injection, binds to proteins and is quickly eliminated from the body before a therapeutic effect can be achieved. A related effort was directed to developing a water-soluble camptothecin sodium salt. Unfortunately, the water-soluble sodium salt of camptothecin remained too toxic for clinical application (Gottlieb et al., 1970 Cancer Chemother. Rep. 54, 461; Moertel et al., 1972 ibid. 56, 95; Gottlieb et al., 1972 ibid. 56, 103).

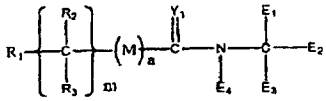
Commonly-assigned PCT publication WO96/23794 describes bis-conjugates in which one equivalent of the hydroxyl-containing drug is attached to each terminal of the polymer. In spite of this advance, techniques which would further increase the payload of the polymer have been sought.

Thus, there continues to be a need to provide additional technologies for forming prodrugs of therapeutic moieties such as camptothecin and related analogs. The present invention addresses this need.

SUMMARY OF THE INVENTION

In one aspect of the invention, compounds of Formula (I) are provided:

(I)



wherein:

R_1 is a polymeric residue;

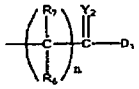
Y_1 is O, S or NR_4;

M is O, S or NR_5;

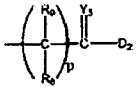
(m) is zero or a positive integer, preferably 1 or 2;

(a) is zero or one;

E_1 is



E_{2-4} are independently H, E_1 or



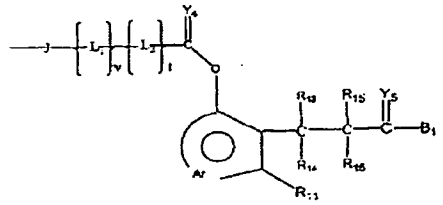
(n) and (p) are independently 0 or a positive integer;

Y_{2-3} are independently O, S or NR_{10};

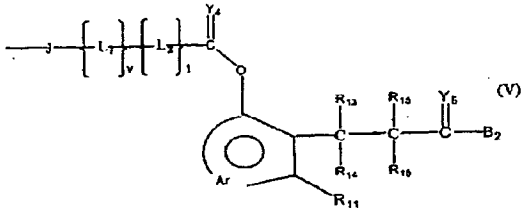
R_{2-10} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-6} cycloalkyls, C_{1-6} substituted alkyls, C_{3-6} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{3-6} heteroalkyls, substituted C_{1-6} hetero-

alkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

D_1 and D_2 are independently OH,



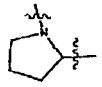
(IV)



(V)

or additional branching groups described below.

Within formulae (IV) and (V), (v) and (t) are independently 0 or a positive integer up to about 6 and preferably about 1;

J is NR_{12} or 

L_1 and L_2 are independently selected bifunctional linkers;

Y_{4-5} are independently selected from the group consisting of O, S and NR_{17};

R_{11-17} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-7} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted

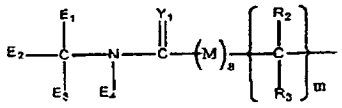
cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-4} heteroalkyls, substituted C_{1-4} heteroalkyls, C_{1-4} alkoxy, phenoxy and C_{1-4} heteroalkoxy;

Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group; and

B_1 and B_2 are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl- or amine-containing moieties.

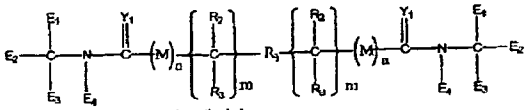
In one particularly preferred aspect of the invention, the polymeric residue is also substituted on the distal portion with a moiety of formula (II) below:

(II)



where all variables are as previously defined. Bifunctional compounds are thus formed when the polymeric residue (R_2) includes both an alpha and an omega terminal linking group so that two, four or more equivalents of a biologically active agent, drug or protein, designated herein as B_1 or B_2 can be delivered. An example of such a bifunctional polymer transport form is illustrated below as formula (III):

(III)



wherein all variables are as described above.

For purposes of the present invention, the term "residue" shall be understood to mean that portion of a biologically active compound which remains after the biologically active compound has undergone a substitution reaction in which the prodrug carrier portion has been attached.

For purposes of the present invention, the term "alkyl" shall be understood to include straight, branched, substituted, e.g. halo-, alkoxy-, and nitro-, C_{1-12} alkyls, C_{3-8} cycloalkyls or substituted cycloalkyls, etc.

For purposes of the present invention, the term "substituted" shall be understood to include adding or replacing one or more atoms contained within a functional group or compound with one or more different atoms.

For purposes of the present invention, substituted alkyls include carboxyalkyls, aminoalkyls, dialkylamines, hydroxyalkyls and mercaptoalkyls; substituted cycloalkyls include moieties such as 4-chlorocyclohexyl; aryls include moieties such as naphthyl; substituted aryls include moieties such as 3-bromophenyl; aralkyls include moieties such as tolyl; heteroalkyls include moieties such as ethylthiophene; substituted heteroalkyls include moieties such as 3-methoxy-thiophene; alkoxy includes moieties such as methoxy; and phenoxy includes moieties such as 3-nitrophenoxy. Halo- shall be understood to include fluoro, chloro, iodo and bromo.

The term "sufficient amounts" for purposes of the present invention shall mean an amount which achieves a therapeutic effect as such effect is understood by those of ordinary skill in the art.

One of the chief advantages of the compounds of the present invention is that the prodrugs have a higher payload per unit of polymer than previous techniques. It is generally preferred that the polymeric first releases the trimethyl lock (TML) based prodrug intermediate by hydrolysis and then the resultant intermediate or "second prodrug" moiety undergoes lactonization to regenerate, for example, a moiety which is either a biologically active compound or a composition comprising a further prodrug. The high payload polymeric conjugates of the present invention are thus unique delivery systems which can contain up to four or a greater number of molecules of a drug.

Methods of making and using the compounds and conjugates described herein are also provided.

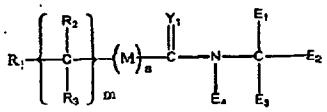
BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-5 schematically illustrate methods of forming compounds of the present invention which are described in the Examples.

DETAILED DESCRIPTION OF THE INVENTION

A. **FORMULA (I)**

In one preferred embodiment of the invention, there are provided compounds of the formula:

(I) 

wherein:

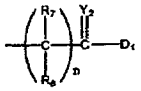
R_1 is a polymeric residue;

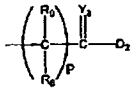
Y_1 is O, S or NR_4;

M is O, S or NR_4;

(a) is zero or one;

(m) is zero or a positive integer;

E_1 is 

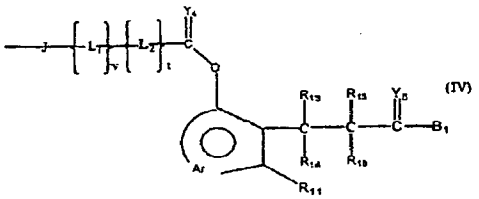
E_{2-4} are independently H, E_1 or 

(n) and (p) are independently 0 or a positive integer;

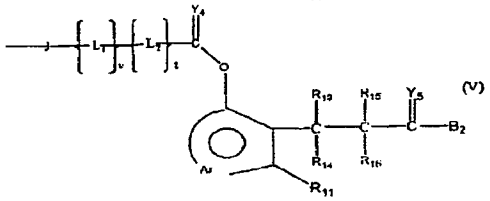
Y_{2-3} are independently O, S or NR_{4a};

R_{2-10} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

D_1 and D_2 are independently OH,

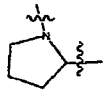


(IV)



(V)

wherein

J is NR_{12} or 

v) and (t) are independently 0 or a positive integer up to about 6 and preferably about 1;

L_1 and L_2 are independently selected bifunctional linkers;

Y_{4-6} are independently selected from the group consisting of O, S and NR_{17};

R_{11-17} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

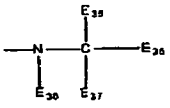
Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group; and

B_1 and B_2 are preferably independently selected from among leaving groups, OH,

residues of hydroxyl-containing moieties or residues of amine-containing moieties.

In another preferred embodiment, D_1 and D_2 are independently selected terminal branching groups of formula (VI)

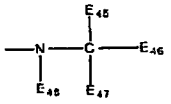
(VI)



wherein:

E_{35-39} are selected from the same group which defines E_{1-4} above, except that within the definition, D_1 and D_2 are changed to D'_1 and D'_2 which are defined below. Within this embodiment, D'_1 and D'_2 can be independently OH, a moiety of formula (IV) or (V), or

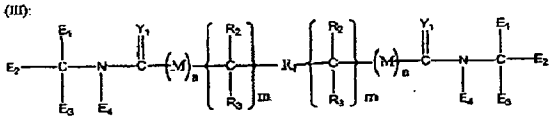
(VII)



wherein E_{45-49} are selected from the same group which defines E_{1-4}, except that within the definition D_1 and D_2 are changed to D''_1 and D''_2 and D''_1 and D''_2 independently OH, formula (IV) or formula (V). As can be appreciated from the above, when the terminal branching is taken to its fullest extent with a bifunctional polymer R_1, up to sixteen (16) equivalents of drug can be loaded onto the polymeric platform.

In those aspects of this embodiment where bis-substituted polymeric residues are desired, some preferred polymeric transport systems of the invention are shown below as formula

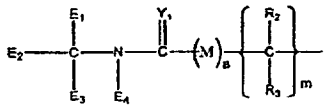
(III):



wherein all variables are as previously described.

The multi-loading polymer transport system of the present invention is based in large part on the polymeric residue designated herein as R_4. Optionally, R_4 includes a capping group A. The polymer capping group A includes, for example, moieties such as hydrogen, CO_2H, C_{1-6} alkyl moieties, and compounds of formula (II) shown below, which forms a bis-system:

(II)



wherein all variables are as previously described. It will be understood and appreciated that the multiple terminal branching described above applies equally in the bis-systems as well.

With regard to the other variables which comprise the formulae of the present invention, the following are preferred:

Y_{1-4} are each oxygen;

R_{2-10} and R_{12} are each preferably hydrogen or lower alkyl, e.g. C_{1-6};

R_{11}, R_{13} and R_{14} are preferably $-CH_3$;

(m) is 1 or 2;

(n) and (p) are each either zero or an integer from 1-4;

(v) is zero or 1;

(t) is 1;

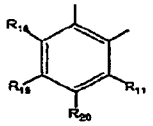
L_1 is $-(CH_2CH_2O)_x-$; and

L_2 is one of $-CH_2-$, $-CH(CH_3)-$, $-(CH_2)_2-$, $-(CH_2)_3-NH-$, $-CH_2C(O)NHCH(CH_3)-$, $-(CH_2)_3-NH-$, $-CH_2C(O)NHCH_2-$, $-(CH_2)_2-NH-C(O)(CH_2)_3NH-$ or $-CH_2C(O)NHCH(CH_2CH(CH_3)_2)-$.

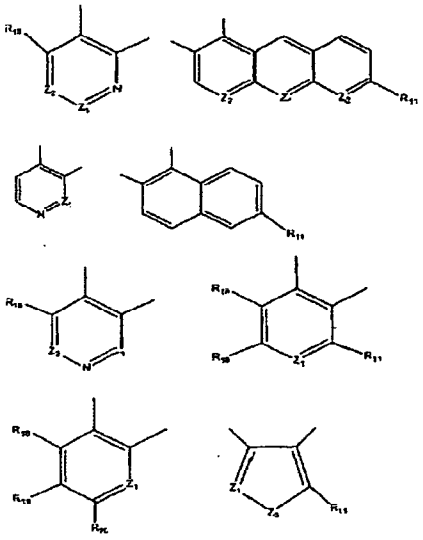
II. DESCRIPTION OF THE Ar MOIETY

Referring to Formula (I), it can be seen that the Ar is a moiety, which when included in Formula (I), forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group. A key feature is that the Ar moiety is aromatic in nature. Generally, to be aromatic, the π electrons must be shared within a "cloud" both above and below the plane of a cyclic molecule. Furthermore, the number of π electrons must satisfy

the Hückel rule ($4n+2$). Those of ordinary skill will realize that a myriad of moieties will satisfy the aromatic requirement of the moiety and thus are suitable for use herein. One particularly preferred aromatic group is:



wherein R_{19-20} are selected from the same group which defines R_{11}. Alternative aromatic groups include:



wherein and Z_1 and Z_2 are independently $CR_{2'}$ or $NR_{2'}$; and Z_3 is O, S or $NR_{2'}$ where $R_{1\&2}$ are selected from the same group as that which defines R_1, or a cyano, nitro, carboxyl, acyl, substituted acyl or carboxyalkyl. Isomers of the five and six-membered rings are also contemplated as well as benzo- and dibenzo- systems and their related congeners are also contemplated. It will also be appreciated by the artisan of ordinary skill that the aromatic rings can optionally be substituted with hetero-atoms such as O, S, $NR_{2'}$, etc. so long as Hückel's rule is obeyed. Furthermore, the aromatic or heterocyclic structures may optionally be substituted with halogen(s) and/or side chains as those terms are commonly understood in the art. However, all structures suitable for Ar moieties of the present invention are capable of allowing the B_1 or B_2-containing moieties and the $(R_{1'})$ moiety to be in an *ortho* arrangement with the same plane.

C. DRUG GENERATION VIA HYDROLYSIS OF THE PRODRUG

The prodrug compounds of the present invention are designed so that the $t_{1/2}$ of hydrolysis is < $t_{1/2}$ elimination in plasma.

The linkages included in the compounds have hydrolysis rates in the plasma of the mammal being treated which is short enough to allow sufficient amounts of the parent compounds, i.e. the amino- or hydroxyl-containing bioactive compound, to be released prior to elimination. Some preferred compounds of the present invention have a $t_{1/2}$ for hydrolysis in plasma ranging from about 5 minutes to about 12 hours. Preferably, the compositions have a plasma $t_{1/2}$ hydrolysis ranging from about 0.5 to about 8 hours and most preferably from about 1 to about 6 hours.

D. SUBSTANTIALLY NON-ANTIGENIC POLYMERS

As stated above, R_1 is a water soluble polymeric residue which is preferably substantially non-antigenic such as a polyalkylene oxide (PAO) or polyethylene glycol (PEG). In preferred aspects of the invention, R_1 further includes the previously mentioned capping group, designated herein as A, which allows a bifunctional or bis-polymer system to be formed.

As an example, the PEG residue portion of the inventive compositions can be selected from the following non-limiting list:

$-C(=Y_3)-(CH_2)_2-O-(CH_2CH_2O)_n-A$,

$-C(=Y_4)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-C(=Y_4)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-C(=Y_4)-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-C(=Y_4)-$,
$-C(=Y_4)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-Y_7-C(=Y_4)-$,
$-C(=Y_4)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{23}-C(=Y_4)-$,
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-O-(CR_{24}R_{25})_e-$, and
$-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{23}-$

wherein Y_4 and Y_7 are independently O, S or NR_{23};

x is the degree of polymerization;

R_{23}, R_{24} and R_{25} are independently selected from among H, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

e and f are independently zero, one or two; and

A is a capping group.

The degree of polymerization for the polymer (x) can be from about 10 to about 2,300. This represents the number of repeating units in the polymer chain and is dependent on the molecular weight of the polymer. The (A) moiety is a capping group as defined herein, i.e. a group which is found on the terminal of the polymer and, in some aspects, can be selected from any of H, NH_2, OH, CO_2H, C_{1-6} alkyls or other PEG terminal activating groups, as such groups are understood by those of ordinary skill.

Also useful are polypropylene glycols, branched PEG derivatives such as those described in commonly-assigned U.S. Patent No. 5,643,575, "star-PEG's" and multi-armed PEG's such as those described in Shearwater Polymers, Inc. catalog "Polyethylene Glycol Derivatives 1997-1998". The disclosure of each of the foregoing is incorporated herein by reference. It will be understood that the water-soluble polymer can be functionalized for attachment to the bifunctional linkage groups if required without undue experimentation.

In a further embodiment R_1 is optionally selected from among one or more of dextran, polyvinyl alcohols, carbohydrate-based polymers, hydroxypropylmethacryl-

amide, polyalkylene oxides, and/or copolymers thereof. See also commonly-assigned U.S. Patent No. 6,153,655, the contents of which are incorporated herein by reference.

In many aspects of the present invention, bis-activated polyethylene glycols are preferred when di- or more substituted polymer conjugates are desired. Alternatively, polyethylene glycols (PEG's), mono-activated, C_{1-4} alkyl-terminated polyalkylene oxides (PAO's) such as mono-methyl-terminated polyethylene glycols (mPEG's) are preferred when mono-substituted polymers are desired.

In order to provide the desired hydrolyzable linkage, mono- or di-acid activated polymers such as PEG acids or PEG diacids can be used as well as mono- or di-PEG amines and mono- or di-PEG diols. Suitable PAO acids can be synthesized by first converting mPEG-OH to an ethyl ester followed by saponification. See also Gehrhardt, H., et al. Polymer Bulletin 18: 487 (1987) and Veronese, F.M., et al., J. Controlled Release 10: 145 (1989). Alternatively, the PAO-acid can be synthesized by converting mPEG-OH into a *t*-butyl ester followed by acid cleavage. See, for example, commonly assigned U.S. Patent No. 5,605,976. The disclosures of each of the foregoing are incorporated by reference herein.

Although PAO's and PEG's can vary substantially in average molecular weight, the polymer portion of the prodrug is at least about 20,000 weight average in most aspects of the invention. Preferably, R_1 has a weight average molecular weight of from about 20,000 to about 100,000 and more preferably from about 25,000 to about 60,000. The average molecular weight of the polymer selected for inclusion in the prodrug must be sufficient so as to provide sufficient circulation of the prodrug before hydrolysis of the linker.

The polymeric substances included herein are preferably water-soluble at room temperature. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained.

As an alternative to PAO-based polymers, effectively non-antigenic materials such as dextran, polyvinyl alcohols, carbohydrate-based polymers, hydroxypropylmethacrylamide (HPMA), and copolymers thereof etc. and the like can be used if the same type of activation is employed as described herein for PAO's such as

PEG. Those of ordinary skill in the art will realize that the foregoing list is merely illustrative and that all polymeric materials having the qualities described herein are contemplated. For purposes of the present invention, "effectively non-antigenic" and "substantially non-antigenic" shall be understood to include all polymeric materials understood in the art as being substantially non-toxic and not eliciting an appreciable immune response in mammals.

It will be clear from the foregoing that other polyalkylene oxide derivatives of the foregoing, such as the polypropylene glycol acids, etc., as well as other bi- functional linking groups are also contemplated.

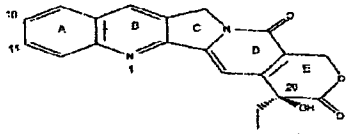
E. PRODRUG CANDIDATES

1. Residues of Hydroxyl-containing Compounds

a. Camptothecin and Related Topoisomerase I Inhibitors

Camptothecin is a water-insoluble cytotoxic alkaloid produced by *Camptotheca acuminata* trees indigenous to China and *nothapodytes foetida* trees indigenous to India. Camptothecin and related compounds and analogs are also known to be potential anticancer or antitumor agents and have been shown to exhibit these activities in vitro and in vivo. Camptothecin and related compounds are also candidates for conversion to the prodrugs of the present invention.

Camptothecin and certain related analogues share the structure:



From this core structure, several known analogs have been prepared. For example, the A ring in either or both of the 10- and 11-positions can be substituted with an OH. The A ring can also be substituted in the 9-position with a straight or branched C_{1-16} alkyl or C_{1-7} alkoxy, optionally linked to the ring by a heteroatom i.e.- O or S. The B ring can be substituted in the 7-position with a straight or branched C_{1-20} alkyl or substituted alkyl-, C_{3-8} cycloakyl, C_{1-20} alkoxy, phenyl alkyl, etc., alkyl carbamate, alkyl

carbazides, phenyl hydrazine derivatives, amino-, aminoalkyl-, aralkyl, etc. Other substitutions are possible in the C, D and E rings. See, for example, U.S. Patent Nos. 5,004,756; 4,943,579; Re 32,518, the contents of which are incorporated herein by reference. Such derivatives can be made using known synthetic techniques without undue experimentation. Preferred camptothecin derivatives for use herein include those which include a 20-OH or another OH moiety which is capable of reacting directly with activated forms of the polymer transport systems described herein or to the linking moiety intermediates, e.g. iminodiacetic acid, etc., which are then attached to a polymer such as PEG.

Reference to camptothecin analogs herein has been made for purposes of illustration and not limitation.

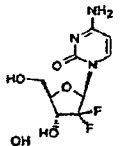
b. Taxanes and Paclitaxel Derivatives

One class of compounds included in the prodrug compositions of the present invention is taxanes. For purposes of the present invention, the term "taxane" includes all compounds within the taxane family of terpenes. Thus, taxol (paclitaxel), 3'-substituted tert-butoxy-carbonyl-amine derivatives (taxoteres) and the like as well as other analogs which are readily synthesized using standard organic techniques or are available from commercial sources such as Sigma Chemical of St. Louis, Missouri are within the scope of the present invention. These derivatives have been found to be effective anti-cancer agents. Numerous studies indicate that the agents have activity against several malignancies. To date, their use has been severely limited by, among other things, their short supply, poor water solubility and a tendency to cause hypersensitivity. It is to be understood that other taxanes including the 7-aryl-carbamates and 7-carbazates disclosed in commonly assigned U.S. Patent Nos. 5,622,986 and 5,547,981 can also be included in the prodrugs of the present invention. The contents of the foregoing U.S. patents are incorporated herein by reference. Paclitaxel is a preferred taxane.

c. Additional Biologically-Active Moieties.

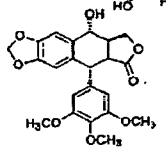
In addition to the foregoing molecules, the prodrug formulations of the present invention can be prepared using many other compounds. For example, biologically-active compounds such as bis-PEG conjugates derived from compounds such as

gemcitabine:



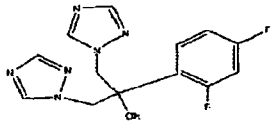
or

podophyllotoxin:

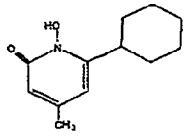


or

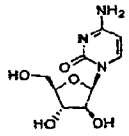
triazole-based antifungal agents such as fluconazole:



or ciclopirox:



or Ara-C:



The parent compounds selected for prodrug forms need not be substantially water-insoluble, although the polymer-based prodrugs of the present invention are especially well suited for delivering such water-insoluble compounds. Other useful parent compounds include, for example, certain low molecular weight biologically active proteins, enzymes and peptides, including peptide glycans, as well as other anti-tumor agents; cardiovascular agents such as forskolin; anti-neoplastics such as combretastatin, vinblastine, daxorubicin, maytansine, etc.; anti-infectives such as vancomycin, erythromycin, etc.; anti-fungals such as nystatin, amphotericin B, triazoles, papulocandins, pneumocandins, echinocandins, polyoxins, nikkomycins, pradimicins, benanomicins, etc. see, "Antibiotics That Inhibit Fungal Cell Wall Development" Annu. Rev. Microbiol. 1994, 48:471-97, the contents of which are incorporated herein by reference; anti-anxiety agents, gastrointestinal agents, central nervous system-activating agents, analgesics, fertility or contraceptive agents, anti-inflammatory agents, steroidal agents, anti-urogenic agents, cardiovascular agents, vasodilating agents, vasoconstricting agents and the like.

The foregoing is illustrative of the biologically active moieties which are suitable for the prodrugs of the present invention. It is to be understood that those biologically active materials not specifically mentioned but having suitable ester-forming groups, i.e. hydroxyl moieties, are also intended and are within the scope of the present invention. It is also to be understood that the prodrug conjugates of the present invention may also include minor amounts of compounds containing not only one equivalent of drug and polymer but also a moiety which does not effect bioactivity in vivo. For example, it has been found that in some instances, in spite of reacting diacids with drug molecules having a single linkage point, the reaction conditions do not provide quantitative amounts of prodrugs with two equivalents of drug per polymer. By-products of the reactants can sometimes be formed such as acyl ureas if carbodiimides are used.

2. Residues of Amine-containing Compounds

In some aspects of the invention, B_1 or B_2 is a residue of an amine-containing compound, a non-limiting list of such suitable compounds include residues of organic compounds, enzymes, proteins, polypeptides, etc. Organic compounds include, without limitation, moieties such as anthracycline compounds including daunorubicin, doxurubicin; *p*-aminoanilline mustard, melphalan, Ara-C (cytosine arabinoside) and

related anti-metabolite compounds, e.g., gemcitabine, etc. Alternatively, B can be a residue of an amine-containing cardiovascular agent, anti-neoplastic, anti-infective, anti-fungal such as nystatin and amphotericin B, anti-anxiety agent, gastrointestinal agent, central nervous system-activating agent, analgesic, fertility agent, contraceptive agent, anti-inflammatory agent, steroidal agent, anti-uremic agent, vasodilating agent, vasoconstricting agent, etc.

In a preferred aspect of the invention, the amino-containing compound is a biologically active compound that is suitable for medicinal or diagnostic use in the treatment of animals, e.g., mammals, including humans, for conditions for which such treatment is desired. The foregoing list is meant to be illustrative and not limiting for the compounds which can be modified. Those of ordinary skill will realize that other such compounds can be similarly modified without undue experimentation. It is to be understood that those biologically active materials not specifically mentioned but having suitable amino-groups are also intended and are within the scope of the present invention.

The only limitations on the types of amino-containing molecules suitable for inclusion herein is that there is available at least one (primary or secondary) amine-containing position which can react and link with a carrier portion and that there is not substantial loss of bioactivity after the prodrug system releases and regenerates the parent compound.

It is noted that parent compounds suitable for incorporation into the prodrug compositions of the invention, may themselves be substances/compounds which are not active after hydrolytic release from the linked composition, but which will become active after undergoing a further chemical process/reaction. For example, an anticancer drug that is delivered to the bloodstream by the double prodrug transport system, may remain inactive until entering a cancer or tumor cell, whereupon it is activated by the cancer or tumor cell chemistry, e.g., by an
enzymatic reaction unique to that cell.

3. Leaving Groups

In those aspects where B_1 or B_2 is a leaving group, suitable leaving groups include, without limitations, moieties such as N-hydroxybenzotriazolyl, halogen, N-hydroxyphthalimidyl, p-nitrophenoxy, imidazolyl, N-hydroxysuccinimidyl; thiazolidinyl thione, or other good leaving groups as will be apparent to those of ordinary

skill. The synthesis reactions used and described herein will be understood by those of ordinary skill without undue experimentation.

For example, an acylated intermediate of compound (I) can be reacted with a reactant such as 4-nitrophenyl chloroformate, disuccinimidyl carbonate (DSC), carbonyldiimidazole, thiazolidine thione, etc. to provide the desired activated derivative.

The selective acylation of the phenolic or anilinic portion of the p-hydroxybenzyl alcohol or the p-aminobenzyl alcohol and the o-hydroxybenzyl alcohol or the o-aminobenzyl alcohol can be carried out with, for example, thiazolidine thione activated polymers, succinimidyl carbonate activated polymers, carboxylic acid activated polymers, blocked amino acid derivatives. Once in place, the "activated" form of the PEG prodrug (or blocked prodrug) is ready for conjugation with an amine- or hydroxyl-containing compound.

F. SYNTHESIS OF THE POLYMERIC PRODRUG TRANSPORT SYSTEM

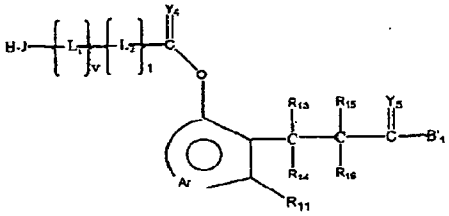
Synthesis of representative polymer prodrugs is set forth in the Examples. Generally, however, in one preferred method of preparing the prodrug transport systems, the polymer residue is first attached to the branching groups. Separately, the biologically active moiety or drug, e.g. Drug-OH or Drug-NH_2 (B_1 or B_2 of formula I) is attached to the TML component which may also include a bifunctional spacer thereon at point of attachment to the polymer. Next, the polymeric residue containing the terminal branches is reacted with the drug-TML portion under conditions sufficient to form the final product.

Attachment of the bifunctional spacer containing the TML-Drug component to the polymer portion is preferably carried out in the presence of a coupling agent. A non-limiting list of suitable coupling agents include 1,3-diisopropylcarbodiimide (DIPC), any suitable dialkyl carbodiimides, 2-halo-1-alkyl-pyridinium halides, (Mukaiyama reagents), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDC), propane phosphonic acid cyclic anhydride (PPACA) and phenyl dichlorophos-phates, etc. which are available, for example from commercial sources such as Sigma-Aldrich Chemical, or synthesized using known techniques.

Preferably the substituents are reacted in an inert solvent such as methylene chloride, chloroform, DMF or mixtures thereof. The reaction also preferably is conducted

in the presence of a base, such as dimethylaminopyridine, diisopropylethylamine, pyridine, triethylamine, etc. to neutralize any acids generated and at a temperature from 0°C up to about 22°C (room temperature).

More particularly, one method of preparing a polymeric transport system includes reacting a compound of the formula (VIII):



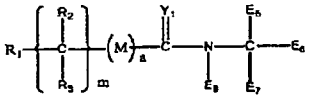
(VIII)

wherein all variables are as previously defined and

B'$_1$ is a residue of a hydroxyl- or an amine-containing moiety;

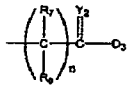
with a compound of the formula (IX):

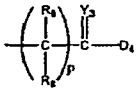
(IX)



wherein

R_1 is a polymeric residue; Y_1 is O, S or NR_4; M is O, S or NR_4; (a) is zero or one; (m) is 0 or a positive integer; Y_{2-5} are independently O, S or NR_{10}; and R_{2-5} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-3} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

E_4 is 



E_{5-8} are independently H, E_3 or

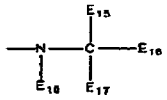
wherein D_3 and D_4 are independently OH or a leaving group which is capable of reacting with an unprotected amine or hydroxyl or a terminal branching group;

(m) and (p) are independently 0 or a positive integer;

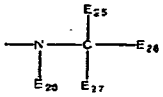
$Y_{2,3}$ are independently O, S or NR_{50}; and

R_{4-10} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-6} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

In further aspects of the method, D_3 and D_4 are independently selected terminal branching groups of formula (X)

(X) 

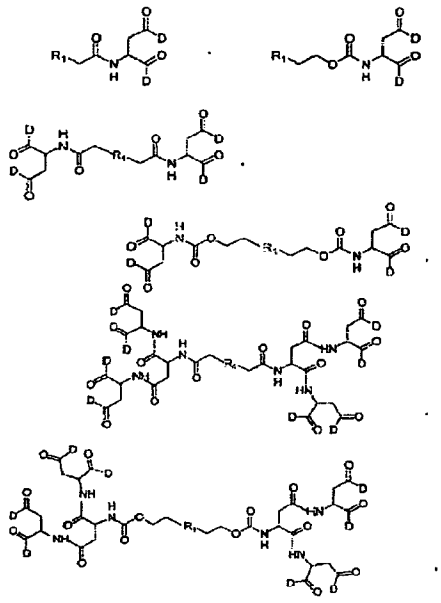
where E_{15-18} are selected from the same group which defines E_{5-8}, except that D_3 and D_4 are changed to D'_3 and D'_4 which are defined below. Within this embodiment, D'_3 and D'_4 can be independently OH, a moiety of formula (IV) or (V), or (XI)

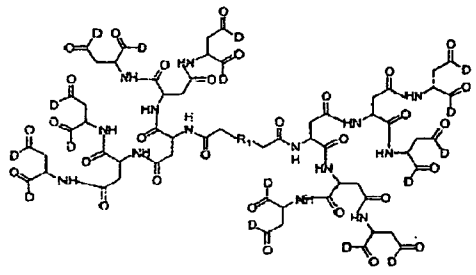
(XI) 

wherein E_{25-29} are selected from the same group which defines E_{5-8}, except that D_3 and D_4 are changed to D''_3 and D''_4 which are defined as being independently OH or a leaving group which is capable of reacting with an unprotected amine or hydroxyl.

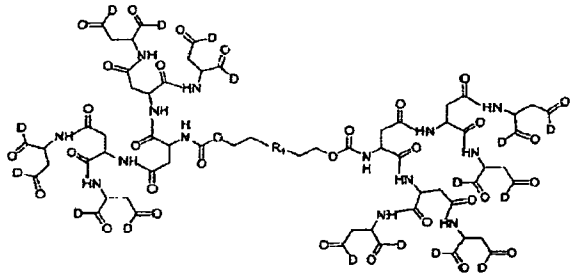
Such synthetic techniques allow up to sixteen (16) equivalents of carboxylic acid or activated carboxylic acid, for example, to be attached. As shown in the preferred structures herein, PEG residues with terminally branched multi-acids are preferred aspects of the invention.

Regardless of the synthesis selected, some of the preferred compounds which result from the synthesis techniques described herein include:

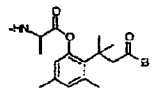


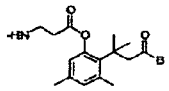


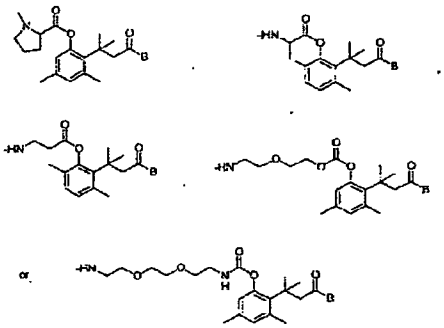
and



wherein R_1 is a polymer residue such as a PAO or PEG and D is OH, formula (IV) or (V).
Preferably, D is



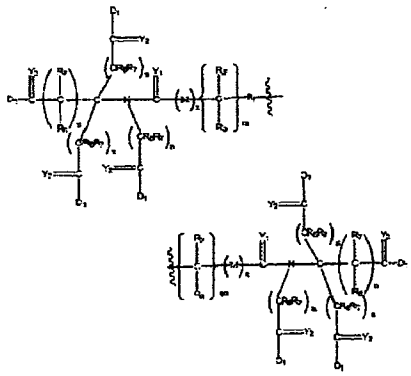




or

where B is a residue of an amine or a hydroxyl- containing drug.

In another preferred aspect of the invention, the compounds of the present invention are of formula (XII):



wherein all variables are as previously defined above.

G. *IN VIVO* DIAGNOSTICS

A further aspect of the invention provides the conjugates of the invention optionally prepared with a diagnostic tag linked to the transport enhancer described above, wherein the tag is selected for diagnostic or imaging purposes. Thus, a suitable tag is prepared by linking any suitable moiety, e.g., an amino acid residue, to any art-standard emitting isotope, radio-opaque label, magnetic resonance label, or other non-radioactive isotopic labels suitable for magnetic resonance imaging, fluorescence-type labels, labels exhibiting visible colors and/or capable of fluorescing under ultraviolet, infrared or electrochemical stimulation, to allow for imaging tumor tissue during surgical procedures, and so forth. Optionally, the diagnostic tag is incorporated into and/or linked to a conjugated therapeutic moiety, allowing for monitoring of the distribution of a therapeutic biologically active material within an animal or human patient.

In a still further aspect of the invention, the inventive tagged conjugates are readily prepared, by art-known methods, with any suitable label, including, e.g., radioisotope labels. Simply by way of example, these include ^{131}Iodine, ^{125}Iodine, ^{99m}Technetium and/or ^{111}Indium to produce radioimmunoscintigraphic agents for selective uptake into tumor cells, *in vivo*. For instance, there are a number of art-known methods of linking peptide to Tc-99m, including, simply by way of example, those shown by U.S. Patent Nos. 5,328,679; 5,888,474; 5,997,844; and 5,997,845, incorporated by reference herein.

Broadly, for anatomical localization of tumor tissue in a patient, the conjugate tag is administered to a patient or animal suspected of having a tumor. After sufficient time to allow the labeled immunoglobulin to localize at the tumor site(s), the signal generated by the label is detected, for instance, visually, by X-ray radiography, computerized transaxial tomography, MRI, by instrumental detection of a luminescent tag, by a photo scanning device such as a gamma camera, or any other method or instrument appropriate for the nature of the selected tag.

The detected signal is then converted to an image or anatomical and/or physiological determination of the tumor site. The image makes it possible to locate the tumor *in vivo* and to devise an appropriate therapeutic strategy. In those embodiments

where the tagged moiety is itself a therapeutic agents, the detected signal provides evidence of anatomical localization during treatment, providing a baseline for follow-up diagnostic and therapeutic interventions.

II. METHODS OF TREATMENT

Another aspect of the present invention provides methods of treatment for various medical conditions in mammals. The methods include administering to the mammal in need of such treatment, an effective amount of a prodrug, such as a multi-loaded Ara-C-PEG conjugates, which has been prepared as described herein. The compositions are useful for, among other things, treating neoplastic disease, reducing tumor burden, preventing metastasis of neoplasms and preventing recurrences of tumor/neoplastic growths in mammals.

The amount of the prodrug administered will depend upon the parent molecule included therein. Generally, the amount of prodrug used in the treatment methods is that amount which effectively achieves the desired therapeutic result in mammals. Naturally, the dosages of the various prodrug compounds will vary somewhat depending upon the parent compound, rate of in vivo hydrolysis, molecular weight of the polymer, etc. In general, however, prodrug taxanes are administered in amounts ranging from about 5 to about 500 mg/m^2 per day, based on the amount of the taxane moiety. Camptothecin prodrugs are also administered in amounts ranging from about 5 to about 500 mg/m^2 per day.. The range set forth above is illustrative and those skilled in the art will determine the optimal dosing of the prodrug selected based on clinical experience and the treatment indication. Actual dosages will be apparent to the artisan without undue experimentation.

The prodrugs of the present invention can be included in one or more suitable pharmaceutical compositions for administration to mammals. The pharmaceutical compositions may be in the form of a solution, suspension, tablet, capsule or the like, prepared according to methods well known in the art. It is also contemplated that administration of such compositions may be by the oral and/or parenteral routes depending upon the needs of the artisan. A solution and/or suspension of the composition may be utilized, for example, as a carrier vehicle for injection or infiltration of the composition by any art known methods, e.g., by intravenous, intramuscular, subdermal injection and the like.

Such administration may also be by infusion into a body space or cavity, as well as by inhalation and/or intranasal routes. In preferred aspects of the invention, however, the prodrugs are parenterally administered to mammals in need thereof.

I. EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention. The underlined and bold-faced numbers recited in the Examples correspond to those shown in **Figures 1-5**.

General. All reactions were run under an atmosphere of dry nitrogen or argon. Commercial reagents were used without further purification. All PEG compounds were dried under vacuum or by azeotropic distillation (toluene) prior to use. ^1H spectra were obtained with a JEOL FT NMR System JNM GSX-270 instrument using deuteriochloroform as solvent unless specified. ^{13}C NMR spectra were obtained at 67.80 MHz on the JNM GSX-270. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) and coupling constants (J values) are given in hertz (Hz). All PEG conjugated compounds were dissolved (~15 mg/mL) in sterile saline (0.9%) for injection prior to *in vivo* drug treatments and were given as their ara-C equivalents (absolute amount of ara-C given).

HPLC Method. Analytical HPLC's were performed using a C8 reversed phase column (Beckman, ultrasphere) under isocratic conditions with an 80:20 mixture (v/v) of methanol-water as mobile phase. Peak elutions were monitored at 254 nm using a UV detector. To detect the presence of any free PEG and also to confirm the presence of PEGYLATED product, an evaporative light scattering detector (ELSD), Model PL-EMD 950 (Polymer Laboratories), was employed. Based on ELSD and UV analysis, all the final PEGylated products were free of native drug and were ≥ 95% pure by HPLC.

Analysis of Ara-C Content in PEG Derivatives. For the determination of the ara-C content in PEG derivatives, *N*⁴-acetylcytidine was used as a model. The UV absorbance of *N*⁴-acetylcytidine in H_2O was determined at 257 nm for six different concentrations ranging from 0.01 μmol/mL to 0.05 μmol/mL. From the standard plot of absorbance *vs.* concentration, the absorption coefficient, ε, of *N*⁴-acetylcytidine was calculated to be 36.4 (O.D. at 257 nm for 1 mg/mL with 1.0 cm light path). PEGylated ara-C derivatives were

dissolved in H_2O at an approximate concentration of 0.015 μmol/mL (based on a MW of 40 kDa) and the UV absorbance of these compounds at 257 nm was determined. Using this value and employing the absorption coefficient, ε, obtained from the above, the concentration of ara-C in the sample was determined. Dividing this value by the sample concentration provided the percentage of ara-C in the sample.

Analysis of Melphalan Content in PEG Derivatives. For the determination of the melphalan content in PEG derivatives, melphalan was used as a standard. The UV absorbance of melphalan in DMF-H_2O (9:1, v/v) was determined at 264 nm for five different concentrations ranging from 0.02 μmol/mL to 0.06 μmol/mL. From the standard plot of absorbance vs. concentration, the absorption coefficient, ε, of melphalan was calculated to be 54.6 (O.D. at 264 nm for 1 mg/mL with 1.0 cm light path).

PEGYLATED melphalan derivatives were dissolved in DMF-H_2O (9:1, v/v) at an approximate concentration of 0.013 μmol/mL (based on a MW of 40 kDa) and the UV absorbance of these compounds at 264 nm was determined. Using this value and employing the absorption coefficient, ε, obtained from the above, the concentration of melphalan in the sample was determined. Dividing this value by the sample concentration provided the percentage of melphalan in the sample.

Abbreviations. DCM (dichloromethane), DMAP (4-(dimethylamino)pyridine), EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), HOBT (1-hydroxybenzotriazole), IPA (2-propanol), NMM (*N*-methylmorpholine), TFA (trifluoroacetic acid).

Example 1.

Compound 3a. A mixture of ara-C (1, 1.73 g, 7.12 mmol), 2a (700 mg, 1.78 mmol), HOBT (0.96 g, 7.12 mmol), and EDC·HCl (2.73 g, 14.25 mmol) in anhydrous pyridine (50 mL) was stirred at room temperature for 2 h, the temperature raised to 40 °C and the reaction continued overnight. The solvent was removed, methylene chloride (50 mL) was used to dissolve the mixture followed by washing with water (3 × 30 mL) and then with 0.1 N HCl (2 × 30 mL). The organic layer was dried over anhydrous $MgSO_4$ and the solvent removed *in vacuo* to give the crude product which was purified by silica gel column chromatography (5 to 10% MeOH in DCM) to give 638.8 mg (52%) of 3a as a white solid: ^{1}H NMR δ 1.42, 1.55, 2.17, 2.26, 2.46, 2.79, 3.84, 3.91, 4.14, 4.33, 4.53, 5.49, 6.07, 6.17, 6.52, 6.76, 7.31, 7.67, 8.16, 8.62; ^{13}C NMR δ 17.77, 20.11, 25.36, 28.32, 31.51, 31.96, 39.57, 50.18, 50.45, 61.88, 74.50, 80.15, 85.90, 88.58, 96.25, 122.51,

132.82, 133.34, 136.73, 138.22, 146.57, 149.90, 155.65, 155.96, 162.08, 171.89, 174.06.

Example 2.

Compound 3b. Compound 1 was coupled with 2b using a similar condition as in Example 1 to produce 3b in 54% yield: ^{13}C NMR δ 17.23, 17.92, 18.33, 25.49, 28.32, 31.51, 31.58, 31.99, 32.46, 39.52, 40.09, 50.08, 50.22, 61.72, 74.50, 74.94, 80.11, 80.15, 85.45, 85.90, 88.01, 88.38, 96.25, 122.51, 128.77, 129.03, 129.16, 131.68, 132.82, 136.24, 136.73, 138.22, 146.05, 146.57, 149.90, 155.65, 155.96, 171.85, 171.89, 174.06.

Example 3.

Compound 4a. Compound 3a (638.8 mg, 1.03 mmol) was stirred in anhydrous DCM (6 mL) and TFA (4 mL) at room temperature for 2 h. Ethyl ether was added to the solution to precipitate the crude product which was filtered and washed with ether to give 4a as a white solid (534.5 mg, 82%): ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 3H, $(CH_3)_2CH$) 1.55 (s, 3H, $(CH_3)_2CH$), 1.62 (d, 1 H, J = 8.1 Hz, $(CH_3)_2CH$), 2.22 (s, 3H, CH_3Ar), 2.57 (s, 3H, CH_3Ar), 2.97 (s, 2H, $CH_2C(=O)$), 3.41-4.27 (m, 5 H, ara-C's H-2'-H5'), 6.09 (d, 1H, J = 5.4, ara-C's H-1'), 6.67 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.12 (d, J = 5.4, H-6), 8.05 (d, J = 8.1, H-5), 8.67 (bs, 1H, TFA); ^{13}C NMR (DMSO-d_6) δ 15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53, 49.02, 61.02, 64.94, 74.64, 76.14, 85.74, 86.95, 94.32, 122.32, 132.41, 134.08, 135.67, 138.09, 146.71, 149.20, 154.50, 158.21, 158.72, 162.02, 169.68, 171.87.

Example 4.

Compound 4b. Compound 3b was subjected to the same condition as in Example 3 to give 4b in 82% yield: ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 3H, $(CH_3)_2CH$) 1.55 (s, 3H, $(CH_3)_2CH$), 1.62 (d, 1 H, J = 8.1 Hz, $(CH_3)_2CH$), 2.22 (s, 3H, CH_3Ar), 2.57 (s, 3H, CH_3Ar), 2.97 (s, 2H, $CH_2C(=O)$), 3.41-4.27 (m, 5 H, ara-C's H-2'-H5'), 6.09 (d, 1H, J = 5.4, ara-C's H-1'), 6.67 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.12 (d, J = 5.4, H-6), 8.05 (d, J = 8.1, H-5), 8.67 (bs, 1H, TFA); ^{13}C NMR (DMSO-d_6) δ 15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53, 49.02, 61.02, 64.94, 74.64, 76.14, 85.74, 86.95, 94.32, 122.32, 132.41, 134.08, 135.67, 138.09, 146.71, 149.20, 154.50, 158.21, 158.72, 162.02, 169.68, 171.87.

Example 5.

Compound 6a. A mixture of PEG-aspartic acid (mw. 40,000, 5.3 g, 0.074 mmol), 4a (385.6 mg, 0.74 mmol), NMM (240 mg, 2.38 mmol), HOBT (120.5 mg, 0.89 mmol), and

EDC·HCl (228.4 mg, 1.19 mmol) in anhydrous DCM (50 mL) was stirred at 0 °C for 30 minutes. The reaction was allowed to warm to room temperature and continued for 3 days and filtered. The filtrate was concentrated *in vacuo* and the residue recrystallized from IPA to give 2.7 g (90%) of product. The amount of sra-C in the product measured by UV assay was 2.11 wt%: ^{13}C NMR δ 14.40, 19.22, 24.56, 31.17, 38.26, 38.90, 47.94, 48.67, 49.66, 60.17, 61.12, 61.90, 67.86-70.87 (PEG), 71.70, 74.50, 85.01, 87.53, 95.28, 121.39, 131.18, 132.68, 133.19, 134.77, 137.70, 145.26, 138.93, 155.23, 160.12, 161.56, 168.39, 170.72, 170.92, 171.27, 171.34.

Example 6.

Compound 6b. Compound 4b was subjected to the same condition as in Example 5 to give 6b in 88% yield. The amount of sra-C in the product measured by UV assay was 1.68 wt%: ^{13}C NMR δ 15.12, 16.22, 24.52, 24.73, 29.55, 30.55, 31.15, 38.04, 38.59, 47.66, 49.16, 49.93, 50.18, 60.93, 61.12, 62.90, 69.44-71.59 (PEG), 71.70, 74.50, 84.78, 84.90, 87.53, 94.85, 127.60, 130.20, 135.51, 136.10, 141.70, 145.15, 147.50, 155.00, 161.20, 169.47, 170.62, 170.92, 171.27.

Example 7.

Compound 9. PEG diol (7, 55 g, 1.38 mmol) was azeotroped in toluene over a 2 hour period followed by removal of 200 mL of solvent by rotary evaporation. The solution was cooled to −30 °C and triphosgene (0.544 g, 1.83 mmol) was added as solid followed by anhydrous pyridine (0.434 g, 5.49 mmol), and the reaction mixture stirred at 50 °C for 1 hour. *N*-hydroxyphthalimide (8, 1.12 g, 6.88 mmol) and anhydrous pyridine (0.54 g, 6.88 mmol) were added to the chloroformate mixture and the reaction stirred for a further 2 hours at 50 °C then for 12 hours at room temperature. The reaction mixture was filtered through filter paper and the solvent removed *in vacuo* and the product crystallized from methylene chloride-ethyl ether (1100 mL, 8:2, v/v) to give the product (50.9 g, 92%): ^{13}C NMR δ 123.62, 128.10, 134.55, 152.00, 160.00.

Example 8.

PEG-ene-Asp-O-*t*-Bu (11). Compound 9 (mw. 40,000, 20 g, 0.459 mmol) and aspartic acid di *t*-butyl ester HCl (10, 1.0 g, 3.55 mmol) were dissolved in anhydrous DCM, followed by addition of DMAP (0.433 g, 3.55 mmol). The solution was refluxed overnight followed by precipitation by addition of ethyl ether (1 L). The solid was isolated by filtration and recrystallized from IPA (1 L) twice. The filter cake was washed

with IPA (200 mL) and ether (200 mL) to give 15.6 g (78%) of product after drying at 45 °C *in vacuo*: ^{13}C NMR δ 27.837 ($CH_2CO_2C(CH_3)_3$), 27.991 ($CHCO_2C(CH_3)_3$), 37.752 ($CHCH_2CO_2$), 50.800 (NHCH), 64.212 ($OCH_2CH_2OC(=O)NH$), 81.333 ($CH_2CO_2C(CH_3)_3$), 82.007 ($CHCO_2C(CH_3)_3$), 155.924 ($OCH_2CH_2OC(=O)NH$), 169.674 ($CH_2CO_2C(CH_3)_3$), 169.969 ($CHCO_2C(CH_3)_3$).

Example 9.

PEG-ene-Asp-OH (12). Compound 11 (15 g, 0.375 mmol) was dissolved in DCM (150 mL) followed by the addition of TFA (75 mL). The solution was stirred at room temperature for 2 hours and hexane (500 mL) added to precipitate the solid. The solid was triturated with hexane to remove TFA followed by recrystallization from chilled DCM-ether. The recrystallized solid was redissolved in DCM (150 mL) and washed with water (150 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, concentrated *in vacuo*, and precipitated with ether to give 12.4 g (83%) of product: ^{13}C NMR δ 36.441 ($CHCH_2CO_2$), 50.177 (NHCH), 64.390 ($OCH_2CH_2OC(=O)NH$), 81.333 ($CH_2CO_2C(CH_3)_3$), 82.007 ($CHCO_2C(CH_3)_3$), 156.172 ($OCH_2CH_2OC(=O)NH$), 171.944 ($CH_2CO_2C(CH_3)_3$), 172.211 ($CHCO_2C(CH_3)_3$).

Example 10.

Boc-Asp-Asp-OMe (15). EDC·HCl (2.47 g, 12.86 mmol) was added to a mixture of BocNH-aspartic acid (13, 1 g, 4.29 mmol), aspartic acid dimethyl ester·HCl (14, 1.86 g, 9.43 mmol), and DMAP (2.47 g, 12.86 mmol) in anhydrous DCM (30 mL) and DMF (2 mL) at 0 °C. The mixture was allowed to warm up to room temperature overnight. The mixture was washed with 1N HCl three times and the organic layer was dried over anhydrous $MgSO_4$, followed by removal of the solvent *in vacuo* to give the product (2.0 g, 90%): ^{1}H NMR δ 1.45 (s, 9*H*), 2.62-3.02 (m, 6H, 3 × C*H*), 3.70 (s, 6H, 2 × OCH_3), 3.74 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.50 (bs, 1H, C*H*), 4.85 (m, 2H, 2 × C*H*), 6.05 (d, *J* = 6.95 Hz, 1H, *NH*), 6.98 (d, *J* = 8.05 Hz, 1H, *NH*), 7.57 (d, *J* = 7.69 Hz, 1H, *NH*).

Example 11.

Asp-Asp-OMe (16). Compound 15 (2.0 g, 3.85 mmol) was dissolved in DCM (30 mL) and TFA (15 mL) and the solution was stirred for 2 h at room temperature. The solvent was removed *in vacuo* and the residue was recrystallized twice with DCM-ether to give the product (1.74 g, 87%) as a white solid: ^{13}C NMR δ 35.52, 48.76, 50.12, 51.90, 51.96, 52.65, 114.59, 118.49, 168.43, 170.02, 170.92, 171.17, 171.40, 171.48.

Example 12.

PEG-cmc-Asp-Asp-OMe (17). DMAP (4.5 g, 36.86 mmol) was added to a solution of 9 (mw. 40,000, 74 g, 1.84 mmol) and 16 (9.83 g, 18.43 mmol) in 700mL of anhydrous chloroform. The reaction mixture was refluxed for 24 hours under nitrogen. The reaction was cooled to room temperature and concentrated to ½ volume. Crude product was precipitated with 2.5 L of ether, filtered and recrystallized from 5.5 L of IPA (65°C). The product was filtered and washed twice with fresh IPA, twice with fresh ether, and dried overnight at 40 °C to yield 59.0g (84%) of 17: ^{13}C NMR δ 35.344, 36.931, 48.082, 48.208, 50.835, 51.509, 52.239, 61.045, 63.953, 68.854-72.056, 155.538, 170.102, 170.369, 170.453, 170.734.

Example 13.

PEG-cmc-Asp-Asp-OH (18). Compound 17 (51 g, 1.26 mmol) and $LiOH·H_2O$ (0.8 g, 18.9 mmol) were dissolved in 300 mL of water and the solution stirred overnight at room temperature. The pH of the solution was adjusted to 2.5 by the addition of 1N HCl. The solution was extracted with DCM (3 × 600 mL), the organic layers combined, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from DCM-ether to give the product which was collected by filtration and dried at 40 °C overnight to yield 38 g (54%) of the octa-acid: ^{13}C NMR (D_2O) δ 38.384, 39.704, 51.951, 54.465, 62.934, 67.105, 71.445-74.381 (PEG), 159.772, 173.831, 174.940, 176.359, 176.696.

Example 14.

Mel-OMe (20). Melphalan (19, 1.00 g, 3.28mmol) was suspended in 2,2 dimethoxypropane (65.59 mL, 533.49 mmol). To the suspension was added aqueous HCl (36 %, 3.28 mL) and absolute methanol (4 mL). The mixture was warmed to mild reflux with vigorous stirring until solution started to turn slightly brown, followed by stirring at room temperature for 18 hours. The reaction mixture was concentrated *in vacuo* and the crude product precipitated from the residue with ether. The solid was filtered, washed with ether, and purified by silica gel column chromatography ($CHCl_3$: MeOH = 9:1, v/v) to yield the desired product (0.47g, 45%): ^{13}C NMR δ 39.751, 40.340, 51.912, 53.435, 55.803, 112.124, 126.076, 130.620, 145.033, 175.754.

Example 15.

Boc-TML-β-Met-OMe (22). EDC (0.52 g, 2.70 mmol) and DMAP (0.988 g, 8.10 mmol) were added to a mixture of 21 (0.531 g, 1.35 mmol) and 20 (0.863 g, 2.70 mmol) in

anhydrous DCM (15 mL) and anhydrous DMF (5 mL) at 0 °C in an ice bath. The reaction mixture was stirred at room temperature overnight under nitrogen then concentrated *in vacuo*. The residue was redissolved in DCM (75 mL) and washed three times with 25mL 1N HCl. The organic layer was dried over anhydrous magnesium sulfate, concentrated, and purified by silica gel column chromatography (ethyl acetate:hexane = 7:3, v/v) to yield the desired product (0.757 g, 80.8 %): ^{13}C NMR δ 20.120, 25.306, 28.294, 31.768, 35.427, 35.947, 36.669, 39.505, 40.311, 49.324, 51.959, 53.234, 53.453, 79.467, 112.095, 123.374, 125.169, 130.439, 132.856, 133.427, 136.666, 138.697, 145.091, 149.841, 156.081, 170.885, 172.298.

Example 16.

TML1β-Mel-OMe TFA Salt (23). Compound 22 (0.757 g, 1.09 mmol) was stirred in DCM (5mL) and TFA (2.5 mL) at room temperature for 2 hours. The reaction solution was concentrated, redissolved in minimal DCM, and precipitated with ether. The product was collected by filtration to yield the desired product (0.222g, 35.9 %): ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 20.026, 25.146, 31.738, 31.892, 35.271, 36.219, 39.163, 40.340, 49.006, 52.219, 53.396, 112.073, 123.260, 124.756, 130.377, 133.026, 133.180, 136.815, 138.595, 145.110, 149.283, 171.069, 171.619, 172.630.

Example 17.

PEG-cmc-TML1β-Mel-OMe (24). A mixture of PEG-cmc-Asp-Asp-OH (12, 1.6g, 0.0391mmol), 23 (0.277g, 0.391mmol), EDC (0.076g, 0.391mmol), and DMAP (0.155g, 1.269mmol) in anhydrous DCM (23 mL) and anhydrous DMF (6 mL) was stirred overnight at room temperature under nitrogen. The solution was concentrated *in vacuo* and the residue recrystallized from 130mL IPA to yield the product (1.543g, 92.5 %). The amount of melphalan in the product measured by UV assay was 2.80% wt/wt. ^{13}C NMR δ 19.642, 24.788, 31.175, 34.350, 35.975, 38.817, 39.905, 48.558, 51.553, 52.608, 60.897, 62.331, 65.145-72.878 (PEG), 111.394, 122.761, 124.425, 129.698, 132.105, 132.878, 135.804, 137.737, 144.316, 149.065, 160.432, 170.608, 171.598.

Example 18.

Boc-TML1β-AraC (25). A solution of Ara-C (1, 9.88 g, 40.66 mmol) in anhydrous pyridine (85 mL) was added to a mixture of 21 (4.0 g, 10.17 mmol), HOBT (5.49 g, 40.66 mmol), EDC (15.61 g, 81.32 mmol), and NMM (8.93mL, 8.21g, 81.32mmol, 8eq) in anhydrous pyridine (200 mL). The reaction mixture was stirred for 48 hours at 40 °C

under nitrogen, followed by concentration *in vacuo*. The residue was redissolved in DCM (300 mL), washed three times with water (100 mL) and twice with 0.1N HCl (100 mL). The organic layer was dried over magnesium sulfate, concentrated, and purified by silica gel column chromatography ($CHCl_3$ – MEOH = 9:1, v/v) to yield the desired product (3.26 g, 52 %): ^{13}C NMR δ 20.315, 25.560, 28.522, 31.660, 35.520, 36.200, 39.221, 50.239, 61.719, 75.171, 76.695, 79.635, 85.341, 88.052, 96.435, 122.894, 132.519, 133.190, 136.186, 138.007, 146.222, 149.109, 155.906, 162.193, 171.733.

Example 19.

TML1β-AraC TFA salt (26). Compound 25 (3 g, 4.85 mmol) was dissolved in DCM (15 mL) followed by addition of TFA (7.5 mL) at 0 °C. Reaction mixture was stirred at 0 °C for 1.2 hours and concentrated *in vacuo* in a cool water bath. Residue was precipitated with DCM-ether to yield the desire product (2.37 g, 77 %): ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 20.0, 25.3, 31.5, 31.7, 35.0, 38.9, 50.2, 60.9, 75.1, 75.8, 85.7, 88.1, 94.9, 109.7, 113.5, 117.3, 121.1, 122.5, 132.6, 136.4, 138.4, 148.7, 149.5, 150.1, 159.2, 159.6, 160.1, 160.6, 161.1, 170.6, 172.7

Example 20.

PEG-cmc-Asp-Asp-TML1β-AraC, octamer (27). Compounds 26 and 18 were subjected to the same condition as in Example18 to prepare 27.

Example 21.

In vitro and in vivo data for compounds 6a and 6b.

In this Example, in vivo and in vitro data are presented and compared to unmodified Ara-C.

In Vivo

Athymic nude mice were implanted subcutaneous with a 4-5 mm^3 tissue fragment of LX-1 collected from donor mice. The tumor trocar site was observed twice weekly and measured once palpable. The tumor volume for each mouse was determined by measuring two dimensions with calipers and calculated using the formula: tumor volume = (length x width^2)/2. When tumors reached the average volume of 90 mm^3, the mice were divided into their experimental groups which consisted of unmodified Ara-C and PEG-Ara-C compounds. The mice were sorted to evenly distribute tumor size, grouped into 4 to 6 mice/group, and ear punched for permanent identification. Drugs were administered intravenously q3d x 4 (Day 1, 4, 7 and 10) via the tail vein at an approximate rate of 0.5

Ara-C was dissolved in DMSO and diluted to the appropriate concentration in culture media. The PEG-Ara-C compounds were dissolved in water and diluted to the appropriate concentrations in culture media.

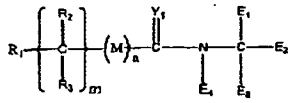
The assays were performed in duplicate in 96-well microtiter cell culture plates. Two fold serial dilution of the compounds were done in the microtiter plates. Cells were detached by incubating with 0.1% Trypsin/Versene at 37°. Trypsin was inactivated by adding the appropriate media for each cell line containing 10% FBS. To each well of the microtiter plates, 10,000 cells were added. After three days, cell growth was measured by addition of a metabolic indicator dye, Alamar Blue, according to the manufacturer's protocol. The IC_{50} value for the test compounds and reference compound are provided above in the Table.

While there have been described what are presently believed to be the preferred embodiments of the invention, those skilled in the art will realize that changes and modifications may be made without departing from the spirit of the invention. It is intended to claim all such changes and modifications as fall within the true scope of the invention.

WHAT IS CLAIMED IS:

1. A compound comprising the formula:

(I)



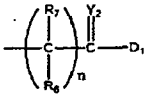
wherein:

R_1 is a polymeric residue;

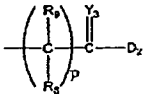
Y_1 is O, S or NR_4;

M is O, S or NR_5;

E_1 is



E_{2-4} are independently H, E_1 or



(a) is zero or one;

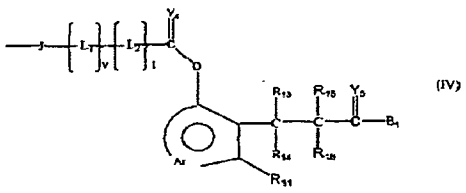
(m) is zero or a positive integer;

(n) and (p) are independently 0 or a positive integer;

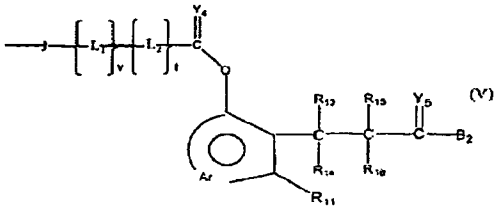
Y_{2-5} are independently O, S or NR_{40};

R_{2-19} are independently selected from the group consisting of hydrogen, C_{1-4} alkyls, C_{3-12} branched alkyls, C_{3-6} cycloalkyls, C_{1-4} substituted alkyls, C_{3-6} substituted cycloalkyls, aryls, substituted aryls, arnlkyls, C_{1-4} heteroalkyls, substituted C_{1-4} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

D_1 and D_2 are independently OH,



(IV)



(V)

or a terminal branching group;

wherein (v) and (t) are independently 0 or a positive integer up to about 6;

J is NR_{13} or 

L_1 and L_2 are independently selected bifunctional linkers;

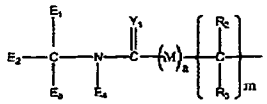
$Y_{4,5}$ are independently selected from the group consisting of O, S and NR_{14};

R_{1-18} are independently selected from the group consisting of hydrogen, C_{1-4} alkyls, C_{3-12} branched alkyls, C_{3-6} cycloalkyls, C_{1-6} substituted alkyls, C_{3-6} substituted cycloalkyls, aryls, substituted aryls, arelkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

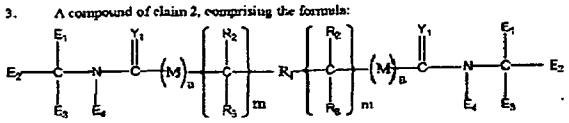
Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group;

B_1 and B_2 are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing moieties or amine-containing moieties.

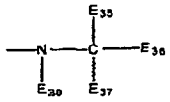
2. The compound of claim 1, wherein R_1 further comprises a capping group A, selected from the group consisting of hydrogen, NH_2, OH, CO_2H, C_{1-6} moieties and



3. A compound of claim 2, comprising the formula:

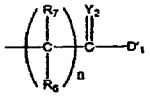


4. The compound of claim 1, wherein said terminal branching group comprises the formula:

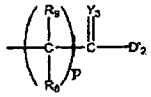


wherein

E_{31} is



E_{32-34} are independently H, E_{35} or



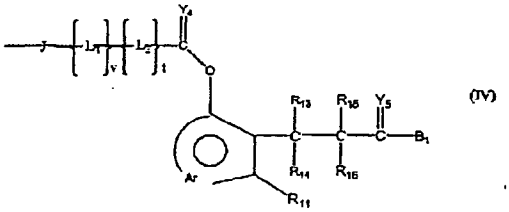
(n) and (p) are independently 0 or a positive integer;

Y_{1-3} are independently O, S or NR_{10};

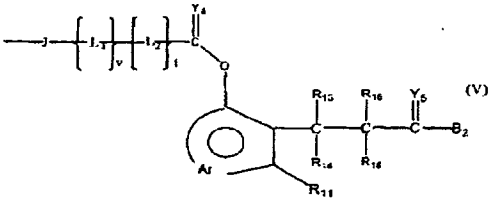
R_{4-10} are independently selected from the group consisting of hydrogen, C_{1-4} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-4} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{3-12} heteroalkyls, substituted C_{1-4} hetero-

alkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

D'_1 and D'_2 are independently OH,



(IV)



(V)

or

(VII)

wherein (v) and (t) are independently 0 or a positive integer up to about 6;

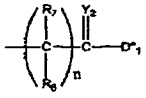
L_1 and L_2 are independently selected bifunctional linkers;

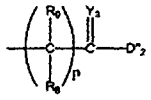
Y_{4-7} are independently selected from the group consisting of O, S and NR_{40};

R_{11-14} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group;

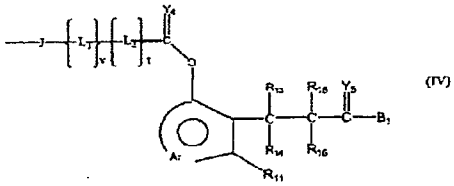
B_1 and B_2 are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing moieties or amine-containing moieties;

E_{x1} is 

E_{x2-x4} are independently H, E_{x1} or 

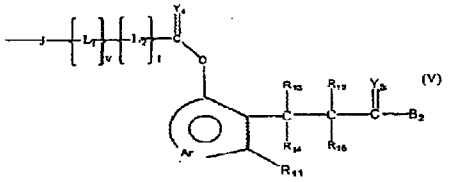
wherein

D''$_1$ and D''$_2$ are independently OH,



(IV)

or



(V)

5. The compound of claim 3, Y_1 is O.

6. The compound of claim 1, wherein R_1 comprises a polyalkylene oxide residue.

7. The compound of claim 6, wherein R_1 comprises a polyethylene glycol residue.

8. The compound of claim 3, wherein R_1 comprises a polyethylene glycol residue.

9. The compound of claim 6, wherein R_1 is selected from the group consisting of
$-C(=Y_e)-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-C(=Y_e)-Y_e-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-C(=Y_e)-NR_{22}-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-(CR_{21}R_{22})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-NR_{22}-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-C(=Y_e)-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-C(=Y_e)-$,
$-C(=Y_e)-Y_e-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-Y_e-C(=Y_e)-$,
$-C(=Y_e)-NR_{22}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{22}-C(=Y_e)-$,
$-(CR_{21}R_{22})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-O-(CR_{21}R_{22})_e-$, and
$-NR_{22}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{22}-$

wherein: Y_e and Y_e are independently O, S or NR_{22};

x is the degree of polymerization;

R_{22}, R_{21} and R_{22} are independently selected from among H, C_{1-6} alkyls, C_{1-6} branched alkyls, C_{1-6} cycloalkyls, C_{1-6} substituted alkyls, C_{1-6} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

e and f are independently zero, one or two; and

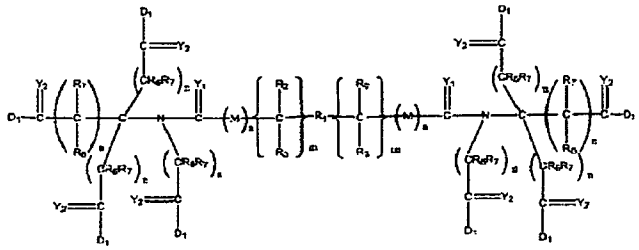
A is a capping group.

10. The compound of claim 9, wherein R_1 comprises $-O-(CH_2CH_2O)_x$ and x is a positive integer so that the weight average molecular weight is at least about 20,000.

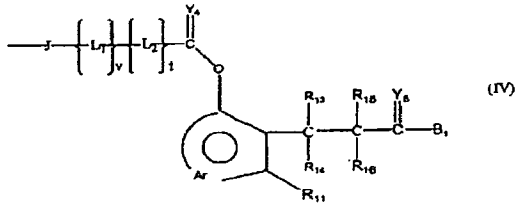
11. The compound of claim 3, wherein R_1 has a weight average molecular weight of from about 20,000 to about 100,000.

12. The compound of claim 3, wherein R_4 has a weight average molecular weight of from about 25,000 to about 60,000.

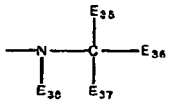
13. A compound of claim 3, comprising the formula



14. The compound of claim 13, wherein D_2 is



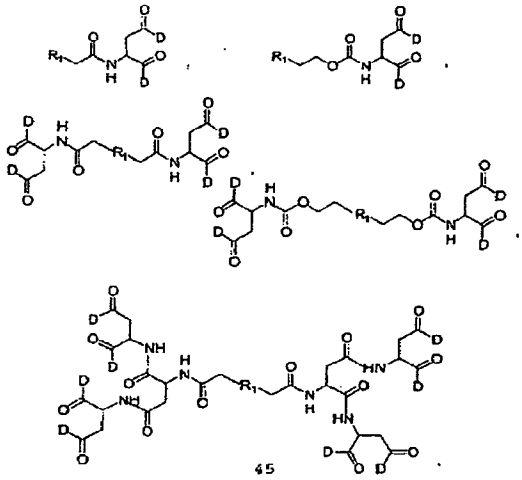
(IV)

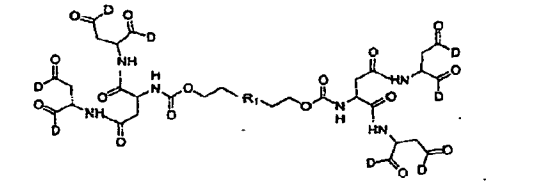
15. The compound of claim 13, wherein D_1 is 

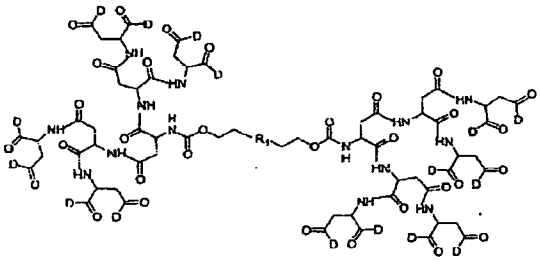
16. The compound of claim 1, wherein L_1 is $(CH_2CH_2O)_2$.

17. The compound of claim 1, wherein L_1 is selected from the group consisting of $-CH_2-$, $-CH(CH_3)-$, $-CH_2C(O)NHCH(CH_3)-$, $-(CH_2)_2-$, $-CH_2C(O)NHCH_2-$, $-(CH_2)_2-NH-$, $-(CH_2)_2-NH-C(O)(CH_2)_2NH-$ and $-CH_2C(O)NHCH(CH_2CH(CH_3)_2)-$.

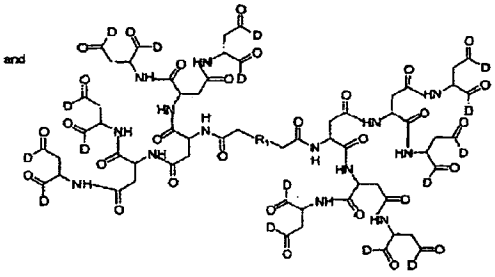
18. A compound of claim 1, selected from the group consisting of:







and



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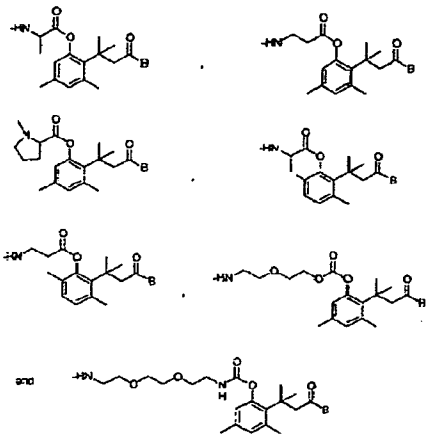
wherein R_1 is a PEG residue and D is selected from the group consisting of:



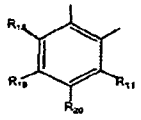
where B is a residue of an amine or a hydroxyl- containing drug.

19. A compound of claim 18, wherein B is a residue of a member of the group consisting of: daunorubicin, doxorubicin; *p*-aminoaniline mustard, melphalan, Ara-C (cytosine arabinoside), leucine-Ara-C, and gemcitabine

20. A method of treatment, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 1, wherein D_1 is a residue of a biologically active moiety.

21. A method of treatment, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 18.

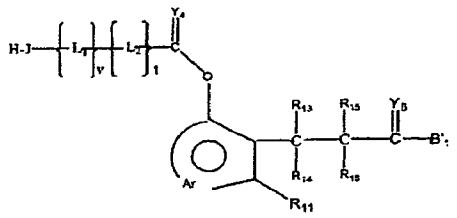
22. The compound of claim 1, wherein Ar comprises the formula:



wherein R_{11} and R_{16-20} are individually selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy.

23. The compound of claim 22, wherein R_{11} and R_{16-20} are each H or CH_3.

24. A method of preparing a polymer conjugate, comprising:
reacting a compound of the formula (VIII):

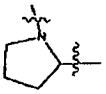


(VIII)

wherein

(v) and (t) are independently 0 or a positive integer up to about 6;

J is NR_{12} or



L_1 and L_2 are independently selected bifunctional linkers;

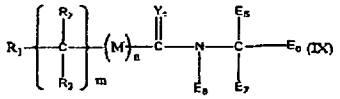
Y_{4-5} are independently selected from the group consisting of O, S and NR_{15};

R_{1-17} are independently selected from the group consisting of hydrogen, C_{1-4} alkyls, C_{3-12} branched alkyls, C_{3-7} cycloalkyls, C_{1-4} substituted alkyls, C_{3-7} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group; and

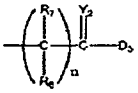
B'_1 is a residue of a hydroxyl- or an amine-containing moiety;

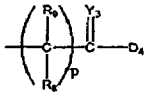
with a compound of the formula (IX):



(IX)

wherein

E_5 is 

E_{1-4} are independently H, E_5 or 

D_3 and D_4 are independently OH, a leaving group which is capable of reacting with an unprotected amine or hydroxyl or a terminal branching group;

R_1 is a polymeric residue;

Y_1 is O, S or NR_{16};

M is O, S or NR_{17};

(n) is zero or one;

(m) is 0 or a positive integer;

(n) and (p) are independently 0 or a positive integer;

Y_{2-5} are independently O, S or NR_{18}; and

R_{9-10} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-6} cycloalkyls, C_{1-6} substituted alkyls, C_{3-6} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

under conditions sufficient to cause a polymeric conjugate to be formed.

Fig. 1

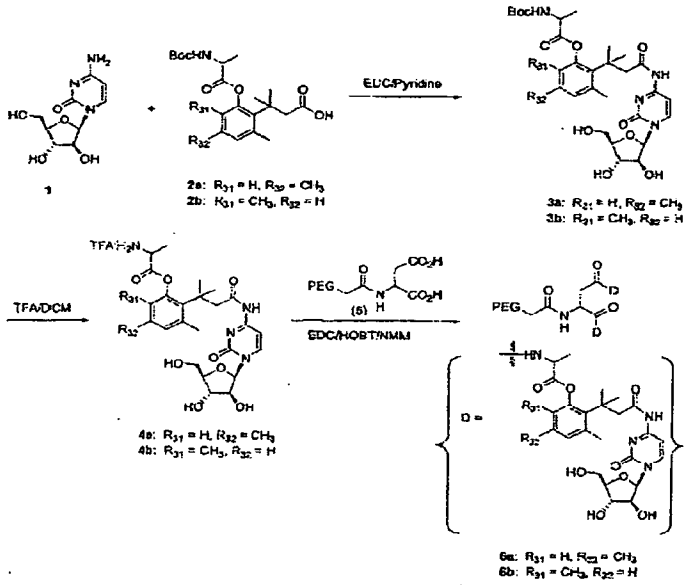

EDC/Pyridine
TFA/DCM
EDC/HOBT/NMM
2a: R31 = H, R32 = CH3
2b: R31 = CH3, R32 = H
3a: R31 = H, R32 = CH3
3b: R31 = CH3, R32 = H
4a: R31 = H, R32 = CH3
4b: R31 = CH3, R32 = H
6a: R31 = H, R32 = CH3
6b: R31 = CH3, R32 = H

Fig. 2

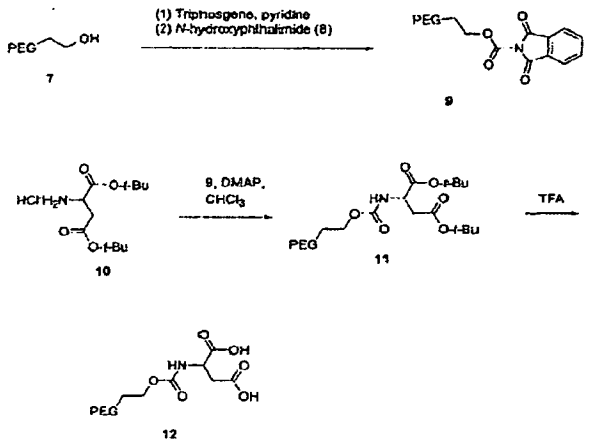

(1) Triphosgene, pyridine
(2) N-hydroxyphthalimide (8)
PEG
OH
7
9
HCl·H2N
O-t-Bu
10
8, DMAP,
CHCl3
HN
11
TFA
12

Fig. 3

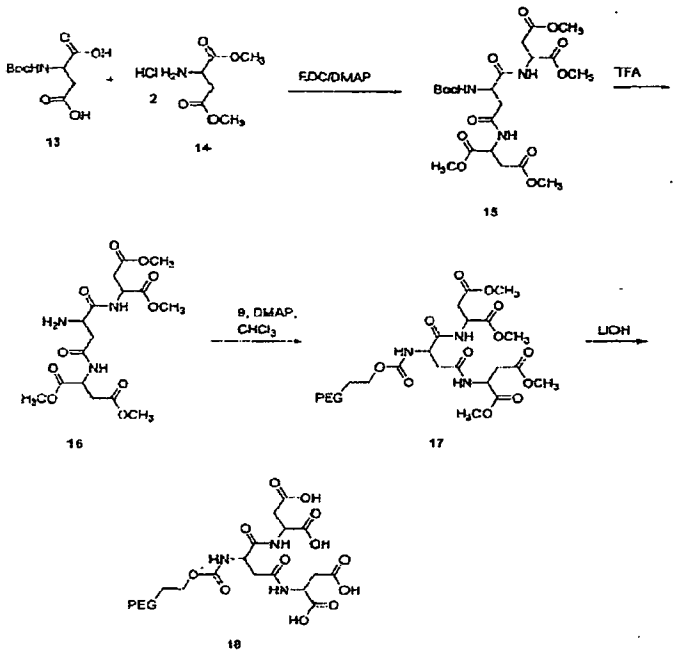


Fig. 4

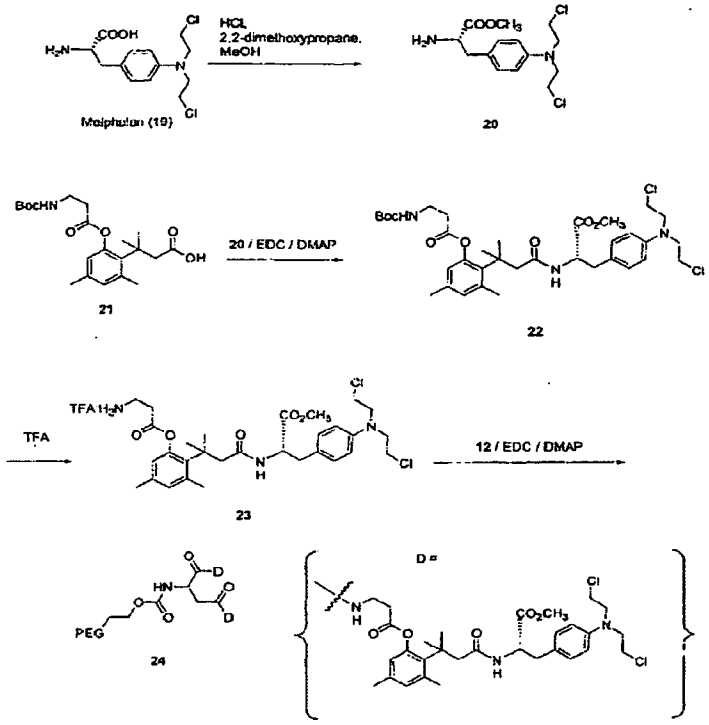
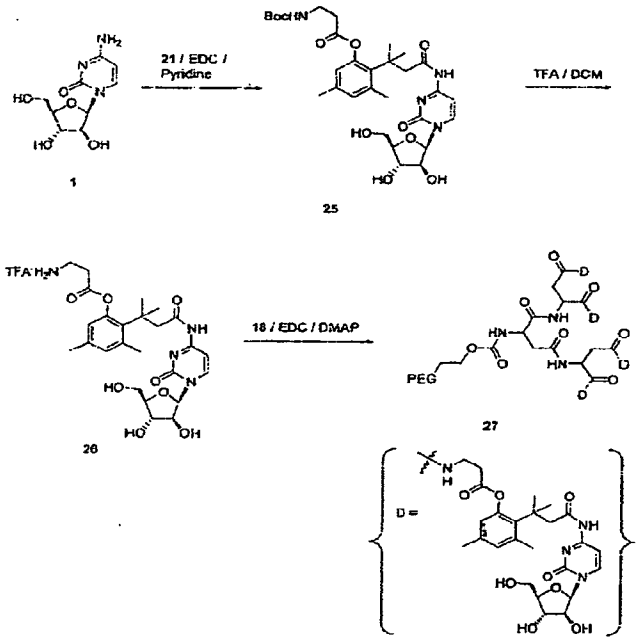

HCl
2,2-dimethoxypropane,
MeOH
Melphalan (19)
20
BocHN
21
20 / EDC / DMAP
22
TFA
TFA·H2N
23
12 / EDC / DMAP
PEG
24
D =

Fig. 5



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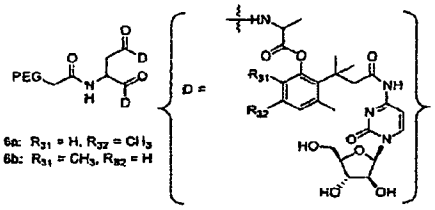
(54) Title: TERMINALLY-BRANCHED POLYMERIC LINKERS AND POLYMERIC CONJUGATES CONTAINING THE SAME



(57) Abstract: Terminally-branched polymeric prodrug platforms capable of high degrees of loading are disclosed. In preferred aspects of the invention, the prodrug platform releases multiple parent compounds after each branch holding the active agent undergoes a benzyl elimination reaction. Methods of preparing the prodrugs and using the same in the treatment of mammals are also disclosed. In one preferred aspect, polymeric conjugates such as formula (I) are provided.

WO 02/066066 A1

TERMINALLY-BRANCHED POLYMERIC LINKERS AND POLYMERIC CONJUGATES CONTAINING THE SAME

TECHNICAL FIELD

The present invention relates to new types of terminally-activated polymeric materials which are useful in forming long-acting conjugates of bioactive materials. In particular, the invention relates to polymeric-based conjugates having increased therapeutic payloads and methods of preparing the same.

BACKGROUND OF THE INVENTION

Over the years, several methods of administering biologically-effective materials to mammals have been proposed. Many medicinal agents are available as water-soluble salts and can be included in pharmaceutical formulations relatively easily. Problems arise when the desired medicinal agent is either insoluble in aqueous fluids or is rapidly degraded in vivo. Alkaloids are often especially difficult to solubilize.

One way to solubilize medicinal agents is to include them as part of a soluble prodrug. Prodrugs include chemical derivatives of a biologically-active parent compound which, upon administration, eventually liberate the parent compound in vivo. Prodrugs allow the artisan to modify the onset and/or duration of action of an agent in vivo and can modify the transportation, distribution or solubility of a drug in the body. Furthermore, prodrug formulations often reduce the toxicity and/or otherwise overcome difficulties encountered when administering pharmaceutical preparations. Typical examples of prodrugs include organic phosphates or esters of alcohols or thiolalcohols. See Remington's Pharmaceutical Sciences, 16th Ed., A. Osol, Ed. (1980), the disclosure of which is incorporated by reference herein.

Prodrugs are often biologically inert or substantially inactive forms of the parent or active compound. The rate of release of the active drug, i.e. the rate of hydrolysis, is

influenced by several factors but especially by the type of bond joining the parent drug to the modifier. Care must be taken to avoid preparing prodrugs which are eliminated through the kidney or reticular endothelial system, etc. before a sufficient amount of hydrolysis of the parent compound occurs.

Incorporating a polymer as part of a prodrug system has been suggested to increase the circulating life of a drug. However, it has been determined that when only one or two polymers of less than about 10,000 daltons each are conjugated to certain biologically active substances such as alkaloid compounds, the resulting conjugates are rapidly eliminated in vivo, especially if a somewhat hydrolysis-resistant linkage is used. In fact, such conjugates are so rapidly cleared from the body that even if a hydrolysis-prone ester linkage is used, not enough of the parent molecule is regenerated in vivo to be therapeutic.

Camptothecin and related biologically active analogs are often poorly water soluble and are examples of substances which would benefit from PEG prodrug technology. A brief overview of some previous work in the field is presented below.

Ohya, et al., J. Bioactive and Compatible Polymers Vol. 10 Jan., 1995, 51-66, disclose doxorubicin-PEG conjugates which are prepared by linking the two substituents via various linkages including esters. The molecular weight of the PEG used, however, is only about 5,000 at most. Thus, the in vivo benefits are not fully realized because the conjugates are substantially excreted prior to sufficient linkage hydrolysis.

U.S. Patent No. 4,943,579 discloses certain simple 20(S)-camptothecin amino acid esters in their salt forms as water soluble prodrugs. The reference does not, however, disclose using an amino acid as part of a linkage which would attach the alkaloid to a relatively high molecular weight polymer in order to form a prodrug. As evidenced by the data provided in Table 2 of the '579 patent, hydrolysis is rapid. Consequently, at physiologic pH, the insoluble base is rapidly generated after injection, binds to proteins and is quickly eliminated from the body before a therapeutic effect can be achieved. A related effort was directed to developing a water-soluble camptothecin sodium salt. Unfortunately, the water-soluble sodium salt of camptothecin remained too toxic for clinical application (Gottlieb et al., 1970 Cancer Chemother. Rep. 54, 461; Moertel et al., 1972 ibid, 56, 95; Gottlieb et al., 1972 ibid, 56, 103).

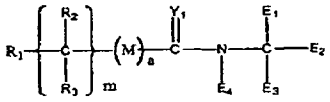
Commonly-assigned PCT publication WO96/23794 describes bis-conjugates in which one equivalent of the hydroxyl-containing drug is attached to each terminal of the polymer. In spite of this advance, techniques which would further increase the payload of the polymer have been sought.

Thus, there continues to be a need to provide additional technologies for forming prodrugs of therapeutic moieties such as camptothecin and related analogs. The present invention addresses this need.

SUMMARY OF THE INVENTION

In one aspect of the invention, compounds of Formula (I) are provided:

(I)



wherein:

R_1 is a polymeric residue;

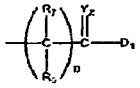
Y_1 is O, S or NR_4;

M is O, S or NR_{15};

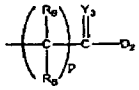
(m) is zero or a positive integer, preferably 1 or 2;

(a) is zero or one;

E is



E_{2-4} are independently H, E_1 or



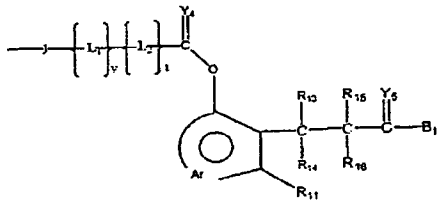
(n) and (p) are independently 0 or a positive integer;

Y_{2-3} are independently O, S or NR_{16};

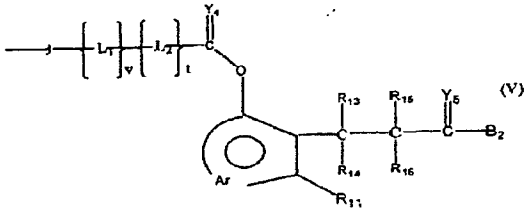
R_{2-16} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{1-6} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} hetero-

alkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

D_1 and D_2 are independently OH,



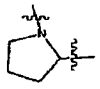
(IV)



(V)

or additional branching groups described below.

Within formulae (IV) and (V), (v) and (t) are independently 0 or a positive integer up to about 6 and preferably about1;

J is NR_{12} or 

L_1 and L_2 are independently selected bifunctional linkers;

Y_{4-5} are independently selected from the group consisting of O, S and NR_{17};

R_{11-17} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-6} cycloalkyls, C_{1-6} substituted alkyls, C_{3-6} substituted

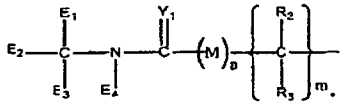
cycloalkyls, aryls, substituted aryls, arylkyls, C_{1-4} heteroalkyls, substituted C_{1-4} heteroalkyls, C_{1-4} alkoxy, phenoxy and C_{1-4} heteroalkoxy;

Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group; and

B_1 and B_2 are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl- or amine-containing moieties.

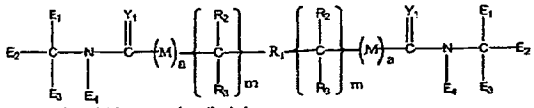
In one particularly preferred aspect of the invention, the polymeric residue is also substituted on the distal portion with a moiety of formula (II) below:

(II)



where all variables are as previously defined. Bifunctional compounds are thus formed when the polymeric residue (R_1) includes both an alpha and an omega terminal linking group so that two, four or more equivalents of a biologically active agent, drug or protein, designated herein as B_1 or B_2 can be delivered. An example of such a bifunctional polymer transport form is illustrated below as formula (III):

(III)



wherein all variables are as described above.

For purposes of the present invention, the term "residue" shall be understood to mean that portion of a biologically active compound which remains after the biologically active compound has undergone a substitution reaction in which the prodrug carrier portion has been attached.

For purposes of the present invention, the term "alkyl" shall be understood to include straight, branched, substituted, e.g. halo-, alkoxy-, and nitro-, C_{1-12} alkyls, C_{3-8} cycloalkyls or substituted cycloalkyls, etc.

For purposes of the present invention, the term "substituted" shall be understood to include adding or replacing one or more atoms contained within a functional group or compound with one or more different atoms.

For purposes of the present invention, substituted alkyls include carboxyalkyls, aminoalkyls, dialkylaminos, hydroxyalkyls and mercaptoalkyls; substituted cycloalkyls include moieties such as 4-chlorocyclohexyl; aryls include moieties such as napthyl; substituted aryls include moieties such as 3-bromophenyl; aralkyls include moieties such as toluyl; heteroalkyls include moieties such as ethylthiophene; substituted heteroalkyls include moieties such as 3-methoxy-thiophene; alkoxy includes moieties such as methoxy; and phenoxy includes moieties such as 3-nitrophenoxy. Halo- shall be understood to include fluoro, chloro, iodo and bromo.

The term "sufficient amounts" for purposes of the present invention shall mean an amount which achieves a therapeutic effect as such effect is understood by those of ordinary skill in the art.

One of the chief advantages of the compounds of the present invention is that the prodrugs have a higher payload per unit of polymer than previous techniques. It is generally preferred that the polymeric first releases the trimethyl lock (TML) based prodrug intermediate by hydrolysis and then the resultant intermediate or "second prodrug" moiety undergoes lactonization to regenerate, for example, a moiety which is either a biologically active compound or a composition comprising a further prodrug. The high payload polymeric conjugates of the present invention are thus unique delivery systems which can contain up to four or a greater number of molecules of a drug.

Methods of making and using the compounds and conjugates described herein are also provided.

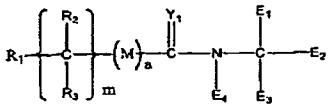
BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1- 5 schematically illustrate methods of forming compounds of the present invention which are described in the Examples.

DETAILED DESCRIPTION OF THE INVENTION

A. FORMULA (I)

In one preferred embodiment of the invention, there are provided compounds of the formula:

(I) 

wherein:

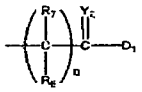
R_1 is a polymeric residue;

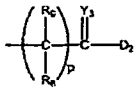
Y_1 is O, S or NR_4;

M is O, S or NR_5;

(a) is zero or one;

(m) is zero or a positive integer;

E_1 is 

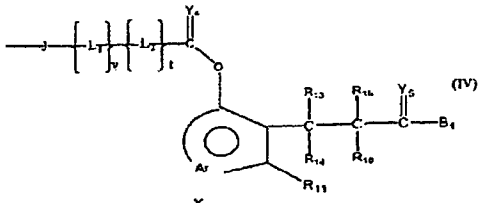
E_{2-4} are independently H, E_1 or 

(n) and (p) are independently 0 or a positive integer;

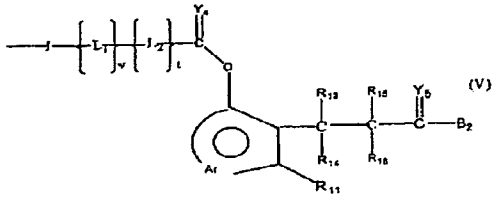
Y_{2-3} are independently O, S or NR_{10};

R_{2-10} are independently selected from the group consisting of hydrogen, C_{1-4} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-4} alkoxy, phenoxy and C_{1-4} heteroalkoxy;

D_1 and D_2 are independently OH.

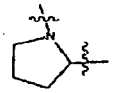


(IV)



(V)

wherein

J is NR_{10} or 

v) and (t) are independently 0 or a positive integer up to about 6 and preferably about1;

L_1 and L_2 are independently selected bifunctional linkers;

Y_{4-6} are independently selected from the group consisting of O, S and NR_{19};

R_{11-17} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, arallcyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

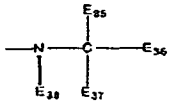
Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group; and

B_1 and B_2 are preferably independently selected from among leaving groups, OH,

residues of hydroxyl-containing moieties or residues of amine-containing moieties.

In another preferred embodiment, D_1 and D_2 are independently selected terminal branching groups of formula (VI)

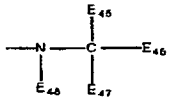
(VI)



wherein:

E_{35-38} are selected from the same group which defines E_{1-4} above, except that within the definition, D_1 and D_2 are changed to D'_1 and D'_2 which are defined below. Within this embodiment, D'_1 and D'_2 can be independently OH, a moiety of formula (IV) or (V), or

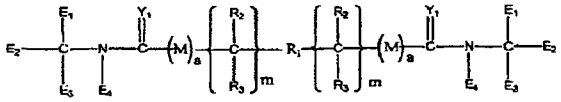
(VII)



wherein E_{45-48} are selected from the same group which defines E_{1-4}, except that within the definition D_1 and D_2 are changed to D''_1 and D''_1 and D''_2 and D''_2 independently OH. formula (IV) or formula (V). As can be appreciated from the above, when the terminal branching is taken to its fullest extent with a bifunctional polymer R_1, up to sixteen (16) equivalents of drug can be loaded onto the polymeric platform.

In those aspects of this embodiment where bis-substituted polymeric residues are desired, some preferred polymeric transport systems of the invention are shown below as formula

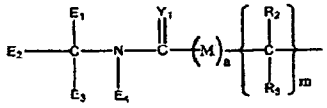
(III):



wherein all variables are as previously described.

The multi-loading polymer transport system of the present invention is based in large part on the polymeric residue designated herein as R_p. Optionally, R_4 includes a capping group A. The polymer capping group A includes, for example, moieties such as hydrogen, CO_2H, C_{1-4} alkyl moieties, and compounds of formula (II) shown below, which forms a bis-system:

(II)



wherein all variables are as previously described. It will be understood and appreciated that the multiple terminal branching described above applies equally in the bis-systems as well.

With regard to the other variables which comprise the formulae of the present invention, the following are preferred:

Y_{1-3} are each oxygen;

R_{2-10} and R_{12} are each preferably hydrogen or lower alkyl, e.g. C_{1-4};

R_{11}, R_{13} and R_{14} are preferably $-CH_3$;

(m) is 1 or 2;

(n) and (p) are each either zero or an integer from 1-4;

(r) is zero or 1;

(t) is 1;

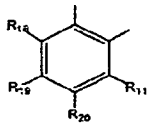
L_1 is $-(CH_2CH_2O)_2-$; and

L_2 is one of $-CH_2-$, $-CH(CH_3)-$, $-(CH_2)_2-$, $-(CH_2)_3-NH-$, $-CH_2C(O)NHCH(CH_3)-$, $-(CH_2)_2-NH-$, $-CH_2C(O)NHCH_2-$, $-(CH_2)_2-NH-C(O)(CH_2)_2NH-$ or $-CH_2C(O)NHCH(CH_2CH(CH_3)_2)-$.

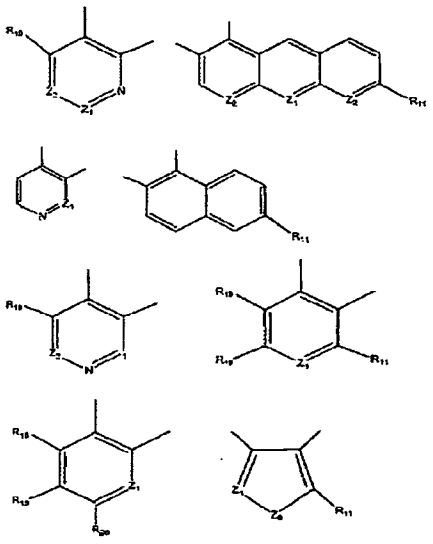
B. DESCRIPTION OF THE Ar MOIETY

Referring to Formula (I), it can be seen that the Ar is a moiety, which when included in Formula (I), forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group. A key feature is that the Ar moiety is aromatic in nature. Generally, to be aromatic, the π electrons must be shared within a "cloud" both above and below the plane of a cyclic molecule. Furthermore, the number of π electrons must satisfy

the Hückel rule (4n+2). Those of ordinary skill will realize that a myriad of moieties will satisfy the aromatic requirement of the moiety and thus are suitable for use herein. One particularly preferred aromatic group is:



wherein R_{16-20} are selected from the same group which defines R_{11}. Alternative aromatic groups include:



wherein and Z_1 and Z_2 are independently CR_{2i} or NR_{4i}; and Z_3 is O, S or NR_{2i} where $R_{4i,2i}$ are selected from the same group as that which defines R_{1i} or a cyano, nitro, carboxyl, acyl, substituted acyl or carbamyalkyl. Isomers of the five and six-membered rings are also contemplated as well as benzo- and dibenzo- systems and their related congeners are also contemplated. It will also be appreciated by the artisan of ordinary skill that the aromatic rings can optionally be substituted with hetero-atoms such as O, S, NR_{2i}, etc. so long as Hückel's rule is obeyed. Furthermore, the aromatic or heterocyclic structures may optionally be substituted with halogen(s) and/or side chains as those terms are commonly understood in the art. However, all structures suitable for Ar moieties of the present invention are capable of allowing the B_1 or B_2-containing moieties and the (R_{1i}) moiety to be in an *ortho* arrangement with the same plane.

C. DRUG GENERATION VIA HYDROLYSIS OF THE PRODRUG

The prodrug compounds of the present invention are designed so that the $t_{1/2}$ of hydrolysis is $< t_{1/2}$ elimination in plasma.

The linkages included in the compounds have hydrolysis rates in the plasma of the mammal being treated which is short enough to allow sufficient amounts of the parent compounds, i.e. the amino- or hydroxyl-containing bioactive compound, to be released prior to elimination. Some preferred compounds of the present invention have a $t_{1/2}$ for hydrolysis in plasma ranging from about 5 minutes to about 12 hours. Preferably, the compositions have a plasma $t_{1/2}$ hydrolysis ranging from about 0.5 to about 8 hours and most preferably from about 1 to about 6 hours.

D. SUBSTANTIALLY NON-ANTIGENIC POLYMERS

As stated above, R_1 is a water soluble polymeric residue which is preferably substantially non-antigenic such as a polyalkylene oxide (PAO) or polyethylene glycol (PEG). In preferred aspects of the invention, R_1 further includes the previously mentioned capping group, designated herein as A, which allows a bifunctional or bis-polymer system to be formed.

As an example, the PEG residue portion of the inventive compositions can be selected from the following non-limiting list:

$-C(=Y_4)-(CH_2)_r-O-(CH_2CH_2O)_s-A$,

$-C(=Y_6)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-C(=Y_6)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-C(=Y_6)-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-C(=Y_6)-$,
$-C(=Y_6)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-Y_7-C(=Y_6)-$,
$-C(=Y_6)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{23}-C(=Y_6)-$,
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-O-(CR_{24}R_{25})_e-$, and
$-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{23}-$

wherein Y_6 and Y_7 are independently O, S or NR_{23};

x is the degree of polymerization;

R_{23}, R_{24} and R_{25} are independently selected from among H, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{3-9} heteroalkyls, substituted C_{1-9} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

e and f are independently zero, one or two; and

A is a capping group.

The degree of polymerization for the polymer (x) can be from about 10 to about 2,300. This represents the number of repeating units in the polymer chain and is dependent on the molecular weight of the polymer. The (A) moiety is a capping group as defined herein, i.e. a group which is found on the terminal of the polymer and, in some aspects, can be selected from any of H, NH_2, OH, CO_2H, C_{1-6} alkyls or other PEG terminal activating groups, as such groups are understood by those of ordinary skill.

Also useful are polypropylene glycols, branched PEG derivatives such as those described in commonly-assigned U.S. Patent No. 5,643,575, "star-PEG's" and multi-armed PEG's such as those described in Shearwater Polymers, Inc. catalog "Polyethylene Glycol Derivatives 1997-1998". The disclosure of each of the foregoing is incorporated herein by reference. It will be understood that the water-soluble polymer can be functionalized for attachment to the bifunctional linkage groups if required without undue experimentation.

In a further embodiment R_1 is optionally selected from among one or more of dextran, polyvinyl alcohols, carbohydrate-based polymers, hydroxypropylmethacryl-

amide, polyalkylene oxides, and/or copolymers thereof. See also commonly-assigned U.S. Patent No. 6,153,655, the contents of which are incorporated herein by reference.

In many aspects of the present invention, bis-activated polyethylene glycols are preferred when di-or more substituted polymer conjugates are desired. Alternatively, polyethylene glycols (PEG's), mono-activated, C_{1-4} alkyl-terminated polyalkylene oxides (PAO's) such as mono-methyl-terminated polyethylene glycols (mPEG's) are preferred when mono-substituted polymers are desired.

In order to provide the desired hydrolyzable linkage, mono- or di-acid activated polymers such as PEG acids or PEG diacids can be used as well as mono- or di-PEG amines and mono- or di-PEG diols. Suitable PAO acids can be synthesized by first converting mPEG-OH to an ethyl ester followed by saponification. See also Gehrhardt, H., et al. Polymer Bulletin 18: 487 (1987) and Veronese, F.M., et al., J. Controlled Release 10; 145 (1989). Alternatively, the PAO-acid can be synthesized by converting mPEG-OH into a t-butyl ester followed by acid cleavage. See, for example, commonly assigned U.S. Patent No. 5,605,976. The disclosures of each of the foregoing are incorporated by reference herein.

Although PAO's and PEO's can vary substantially in average molecular weight, the polymer portion of the prodrug is at least about 20,000 weight average in most aspects of the invention. Preferably, R_1 has a weight average molecular weight of from about 20,000 to about 100,000 and more preferably from about 25,000 to about 60,000. The average molecular weight of the polymer selected for inclusion in the prodrug must be sufficient so as to provide sufficient circulation of the prodrug before hydrolysis of the linker.

The polymeric substances included herein are preferably water-soluble at room temperature. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained.

As an alternative to PAO-based polymers, effectively non-antigenic materials such as dextran, polyvinyl alcohols, carbohydrate-based polymers, hydroxypropylmethacrylamide (HPMA), and copolymers thereof etc. and the like can be used if the same type of activation is employed as described herein for PAO's such as

PEG. Those of ordinary skill in the art will realize that the foregoing list is merely illustrative and that all polymeric materials having the qualities described herein are contemplated. For purposes of the present invention, "effectively non-antigenic" and "substantially non-antigenic" shall be understood to include all polymeric materials understood in the art as being substantially non-toxic and not eliciting an appreciable immune response in mammals.

It will be clear from the foregoing that other polyalkylene oxide derivatives of the foregoing, such as the polypropylene glycol acids, etc., as well as other bi- functional linking groups are also contemplated.

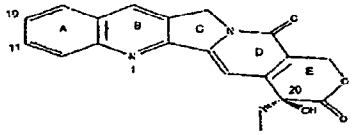
E. PRODRUG CANDIDATES

1. Residues of Hydroxyl-containing Compounds

a. Camptothecin and Related Topoisomerase I Inhibitors

Camptothecin is a water-insoluble cytotoxic alkaloid produced by *Camptotheca acuminata* trees indigenous to China and *nothapodytes foetida* trees indigenous to India. Camptothecin and related compounds and analogs are also known to be potential anticancer or antitumor agents and have been shown to exhibit these activities in vitro and in vivo. Camptothecin and related compounds are also candidates for conversion to the prodrugs of the present invention.

Camptothecin and certain related analogues share the structure:



From this core structure, several known analogs have been prepared. For example, the A ring in either or both of the 10- and 11-positions can be substituted with an OH. The A ring can also be substituted in the 9-position with a straight or branched C_{1-30} alkyl or C_{1-17} alkoxy, optionally linked to the ring by a heteroatom i.e.- O or S. The B ring can be substituted in the 7-position with a straight or branched C_{1-30} alkyl or substituted alkyl-, C_{1-8} cycloalkyl, C_{1-30} alkoxy, phenyl alkyl, etc., alkyl carbamate, alkyl

carbazides, phenyl hydrazine derivatives, amino-, aminoalkyl-, aralkyl, etc. Other substitutions are possible in the C, D and E rings. See, for example, U.S. Patent Nos. 5,004,758; 4,943,579; Re 32,518, the contents of which are incorporated herein by reference. Such derivatives can be made using known synthetic techniques without undue experimentation. Preferred camptothecin derivatives for use herein include those which include a 20-OH or another OH moiety which is capable of reacting directly with activated forms of the polymer transport systems described herein or to the linking moiety intermediates, e.g. iminodiacetic acid, etc., which are then attached to a polymer such as PEG.

Reference to camptothecin analogs herein has been made for purposes of illustration and not limitation.

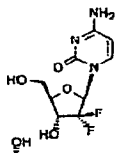
b. Taxanes and Paclitaxel Derivatives

One class of compounds included in the prodrug compositions of the present invention is taxanes. For purposes of the present invention, the term "taxane" includes all compounds within the taxane family of terpenes. Thus, taxol (paclitaxel), 3'-substituted tert-butoxy-carbonyl-amine derivatives (taxoteres) and the like as well as other analogs which are readily synthesized using standard organic techniques or are available from commercial sources such as Sigma Chemical of St. Louis, Missouri are within the scope of the present invention. These derivatives have been found to be effective anti-cancer agents. Numerous studies indicate that the agents have activity against several malignancies. To date, their use has been severely limited by, among other things, their short supply, poor water solubility and a tendency to cause hypersensitivity. It is to be understood that other taxanes including the 7-aryl-carbonates and 7-carbazates disclosed in commonly assigned U.S. Patent Nos. 5,622,986 and 5,547,981 can also be included in the prodrugs of the present invention. The contents of the foregoing U.S. patents are incorporated herein by reference. Paclitaxel is a preferred taxane.

c. Additional Biologically-Active Moieties

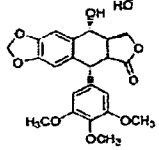
In addition to the foregoing molecules, the prodrug formulations of the present invention can be prepared using many other compounds. For example, biologically-active compounds such as bis-PEG conjugates derived from compounds such as

gemcitabine:



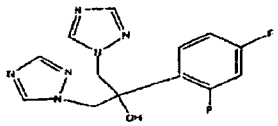
or

podophyllotoxin:

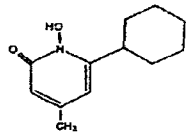


or

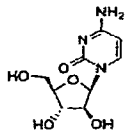
triazole-based antifungal agents such as fluconazole:



or ciclopirox:



or Ara-C:



The parent compounds selected for prodrug forms need not be substantially water-insoluble, although the polymer-based prodrugs of the present invention are especially well suited for delivering such water-insoluble compounds. Other useful parent compounds include, for example, certain low molecular weight biologically active proteins, enzymes and peptides, including peptido glycans, as well as other anti-tumor agents; cardiovascular agents such as forskolin; anti-neoplastics such as combretastatin, vinblastine, doxorubicin, maytansine, etc.; anti-infectives such as vancomycin, erythromycin, etc.; anti-fungals such as nystatin, amphotericin B, triazoles, papulocandins, pneumocandins, echinocandins, polyoxins, nikkomycins, pradimicins, benanomicins, etc. see, "Antibiotics That Inhibit Fungal Cell Wall Development" Annu. Rev. Microbiol. 1994, 48:471-97, the contents of which are incorporated herein by reference; anti-anxiety agents, gastrointestinal agents, central nervous system-activating agents, analgesics, fertility or contraceptive agents, anti-inflammatory agents, steroidal agents, anti-neoplastic agents, cardiovascular agents, vasodilating agents, vasoconstricting agents and the like.

The foregoing is illustrative of the biologically active moieties which are suitable for the prodrugs of the present invention. It is to be understood that those biologically active materials not specifically mentioned but having suitable ester-forming groups, i.e. hydroxyl moieties, are also intended and are within the scope of the present invention. It is also to be understood that the prodrug conjugates of the present invention may also include minor amounts of compounds containing not only one equivalent of drug and polymer but also a moiety which does not effect bioactivity in vivo. For example, it has been found that in some instances, in spite of reacting diacids with drug molecules having a single linkage point, the reaction conditions do not provide quantitative amounts of prodrugs with two equivalents of drug per polymer. By-products of the reactants can sometimes be formed such as acyl ureas if carbodiimides are used.

2. Residues of Amine-containing Compounds

In some aspects of the invention, B_1 or B_2 is a residue of an amine-containing compound, a non-limiting list of such suitable compounds include residues of organic compounds, enzymes, proteins, polypeptides, etc. Organic compounds include, without limitation, moieties such as anthracycline compounds including daunorubicin, doxorubicin; *p*-aminoaniline mustard, melphalan, Ara-C (cytosine arabinoside) and

related anti-metabolite compounds, e.g., gemcitabine, etc. Alternatively, B can be a residue of an amino-containing cardiovascular agent, anti-neoplastic, anti-infective, anti-fungal such as nystatin and amphotericin B, anti-anxiety agent, gastrointestinal agent, central nervous system-activating agent, analgesic, fertility agent, contraceptive agent, anti-inflammatory agent, steroidal agent, anti-uremic agent, vasodilating agent, vasoconstricting agent, etc.

In a preferred aspect of the invention, the amino-containing compound is a biologically active compound that is suitable for medicinal or diagnostic use in the treatment of animals, e.g., mammals, including humans, for conditions for which such treatment is desired. The foregoing list is meant to be illustrative and not limiting for the compounds which can be modified. Those of ordinary skill will realize that other such compounds can be similarly modified without undue experimentation. It is to be understood that those biologically active materials not specifically mentioned but having suitable amino-groups are also intended and are within the scope of the present invention.

The only limitations on the types of amino-containing molecules suitable for inclusion herein is that there is available at least one (primary or secondary) amino-containing position which can react and link with a carrier portion and that there is not substantial loss of bioactivity after the prodrug system releases and regenerates the parent compound.

It is noted that parent compounds suitable for incorporation into the prodrug compositions of the invention, may themselves be substances/compounds which are not active after hydrolytic release from the linked composition, but which will become active after undergoing a further chemical process/reaction. For example, an anticancer drug that is delivered to the bloodstream by the double prodrug transport system, may remain inactive until entering a cancer or tumor cell, whereupon it is activated by the cancer or tumor cell chemistry, e.g., by an
enzymatic reaction unique to that cell.

3. Leaving Groups

In those aspects where B_1 or B_2 is a leaving group, suitable leaving groups include, without limitations, moieties such as N-hydroxybenzotriazolyl, halogen, N-hydroxyphthalimidyl, p-nitrophenoxy, imidazolyl, N-hydroxysuccinimidyl; thiazolidinyl thione, or other good leaving groups as will be apparent to those of ordinary

skill. The synthesis reactions used and described herein will be understood by those of ordinary skill without undue experimentation.

For example, an acylated intermediate of compound (I) can be reacted with a reactant such as 4-nitrophenyl chloroformate, disuccinimidyl carbonate (DSC), carbonyldiimidazole, thiazolidine thione, etc. to provide the desired activated derivative.

The selective acylation of the phenolic or anilinic portion of the p-hydroxybenzyl alcohol or the p-aminobenzyl alcohol and the o-hydroxbenzyl alcohol or the o-aminobenzyl alcohol can be carried out with, for example, thiazolidine thione activated polymers, succinimidyl carbonate activated polymers, carbosylic acid activated polymers, blocked amino acid derivatives. Once in place, the "activated" form of the PEG prodrug (or blocked prodrug) is ready for conjugation with an amine- or hydroxyl-containing compound.

F. SYNTHESIS OF THE POLYMERIC PRODRUG TRANSPORT SYSTEM

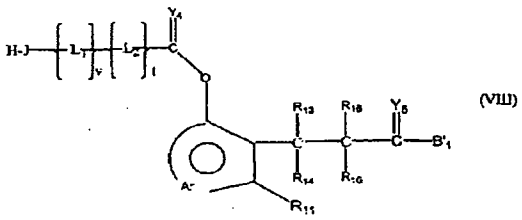
Synthesis of representative polymer prodrugs is set forth in the Examples. Generally, however, in one preferred method of preparing the prodrug transport systems, the polymer residue is first attached to the branching groups. Separately, the biologically active moiety or drug, e.g. Drug-OH or Drug-NH, (B, or B_1 of formula I) is attached to the TML component which may also include a bifunctional spacer thereon at point of attachment to the polymer. Next, the polymeric residue containing the terminal branches is reacted with the drug-TML portion under conditions sufficient to form the final product.

Attachment of the bifunctional spacer containing the TML-Drug component to the polymer portion is preferably carried out in the presence of a coupling agent. A non-limiting list of suitable coupling agents include 1,3-diisopropylcarbodiimide (DIPC), any suitable dialkyl carbodiimides, 2-halo-1-alkyl-pyridinium halides, (Mukaiyama reagents), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDC), propane phosphonic acid cyclic anhydride (PPACA) and phenyl dichlorophos-phates, etc. which are available, for example from commercial sources such as Sigma-Aldrich Chemical, or synthesized using known techniques.

Preferably the substituents are reacted in an inert solvent such as methylene chloride, chloroform, DMF or mixtures thereof. The reaction also preferably is conducted

in the presence of a base, such as dimethylaminopyridine, diisopropylethylamine, pyridine, triethylamine, etc. to neutralize any acids generated and at a temperature from 0°C up to about 22°C (room temperature).

More particularly, one method of preparing a polymeric transport system includes reacting a compound of the formula (VIII):



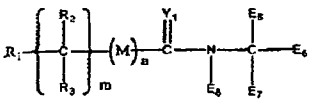
(VIII)

wherein all variables are as previously defined and

B'_1 is a residue of a hydroxyl- or an amine-containing moiety;

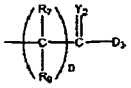
with a compound of the formula (IX):

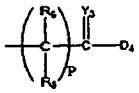
(IX)



wherein

R_1 is a polymeric residue; Y_1 is O, S or NR_8; M is O, S or NR_9; (a) is zero or one; (m) is 0 or a positive integer; Y_{2-3} are independently O, S or NR_{10}; and R_{2-3} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

E_3 is 



E_{4-6} are independently H, E_3 or

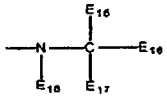
wherein D_3 and D_4 are independently OH or a leaving group which is capable of reacting with an unprotected amine or hydroxyl or a terminal branching group;

(n) and (p) are independently 0 or a positive integer;

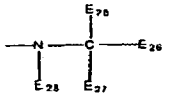
$Y_{2,3}$ are independently O, S or NR_{50}; and

R_{4-50} are independently selected from the group consisting of hydrogen, C_{1-4} alkyls, C_{5-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-4} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

In further aspects of the method, D_3 and D_4 are independently selected terminal branching groups of formula (X)

(X) 

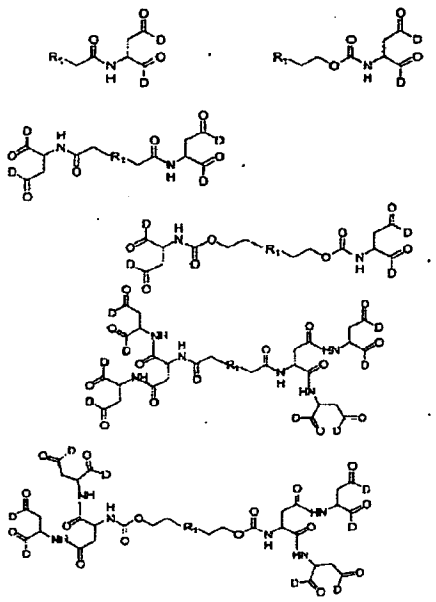
where E_{15-18} are selected from the same group which defines E_{4-6}, except that D_3 and D_4 are changed to D'_3 and D'_4 which are defined below. Within this embodiment, D'_3 and D'_4 can be independently OH, a moiety of formula (IV) or (V), or (XI)

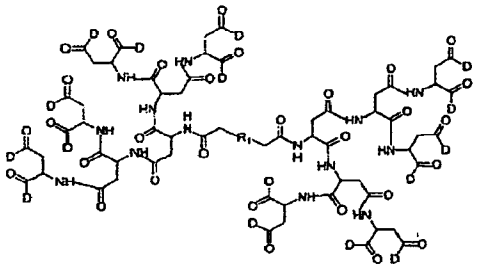
(XI) 

wherein E_{25-28} are selected from the same group which defines E_{15-18}, except that D_3 and D_4 are changed to D''_3 and D''_4 which are defined as being independently OH or a leaving group which is capable of reacting with an unprotected amine or hydroxyl.

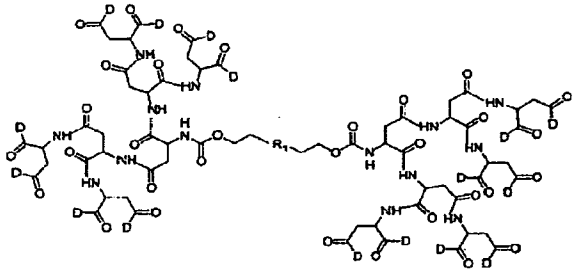
Such synthetic techniques allow up to sixteen (16) equivalents of carboxylic acid or activated carboxylic acid, for example, to be attached. As shown in the preferred structures herein, PEG residues with terminally branched multi-acids are preferred aspects of the invention.

Regardless of the synthesis selected, some of the preferred compounds which result from the synthesis techniques described herein include:



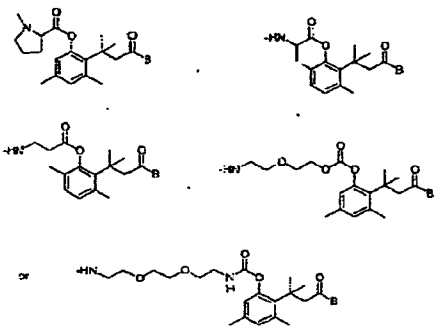


and



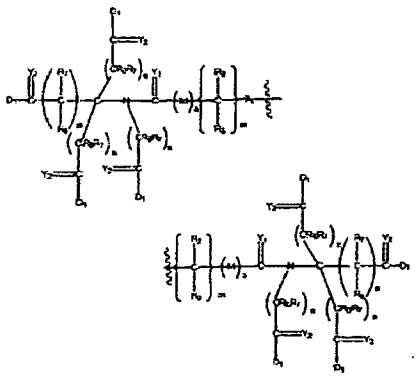
wherein R_1 is a polymer residue such as a PAO or PEG and D is OH. formula (IV) or (V).

Preferably, D is



where B is a residue of an amine or a hydroxyl- containing drug.

In another preferred aspect of the invention, the compounds of the present invention are of formula (XII):



wherein all variables are as previously defined above.

G. *IN VIVO* DIAGNOSTICS

A further aspect of the invention provides the conjugates of the invention optionally prepared with a diagnostic tag linked to the transport enhancer described above, wherein the tag is selected for diagnostic or imaging purposes. Thus, a suitable tag is prepared by linking any suitable moiety, e.g., an amino acid residue, to any art-standard emitting isotope, radio-opaque label, magnetic resonance label, or other non-radioactive isotopic labels suitable for magnetic resonance imaging, fluorescence-type labels, labels exhibiting visible colors and/or capable of fluorescing under ultraviolet, infrared or electrochemical stimulation, to allow for imaging tumor tissue during surgical procedures, and so forth. Optionally, the diagnostic tag is incorporated into and/or linked to a conjugated therapeutic moiety, allowing for monitoring of the distribution of a therapeutic biologically active material within an animal or human patient.

In a still further aspect of the invention, the inventive tagged conjugates are readily prepared, by art-known methods, with any suitable label, including, e.g., radioisotope labels. Simply by way of example, these include ^{131}Iodine, ^{125}Iodine, ^{99m}Technetium and/or ^{111}Indium to produce radioimmunoscintigraphic agents for selective uptake into tumor cells, *in vivo*. For instance, there are a number of art-known methods of linking peptide to Tc-99m, including, simply by way of example, those shown by U.S. Patent Nos. 5,328,679; 5,888,474; 5,997,844; and 5,997,845, incorporated by reference herein.

Broadly, for anatomical localization of tumor tissue in a patient, the conjugate tag is administered to a patient or animal suspected of having a tumor. After sufficient time to allow the labeled immunoglobulin to localize at the tumor site(s), the signal generated by the label is detected, for instance, visually, by X-ray radiography, computerized transaxial tomography, MRI, by instrumental detection of a luminescent tag, by a photo scanning device such as a gamma camera, or any other method or instrument appropriate for the nature of the selected tag.

The detected signal is then converted to an image or anatomical and/or physiological determination of the tumor site. The image makes it possible to locate the tumor *in vivo* and to devise an appropriate therapeutic strategy. In those embodiments

where the tagged moiety is itself a therapeutic agents, the detected signal provides evidence of anatomical localization during treatment, providing a baseline for follow-up diagnostic and therapeutic interventions.

B. METHODS OF TREATMENT

Another aspect of the present invention provides methods of treatment for various medical conditions in mammals. The methods include administering to the mammal in need of such treatment, an effective amount of a prodrug, such as a multi-loaded Ara-C-PEG conjugates, which has been prepared as described herein. The compositions are useful for, among other things, treating neoplastic disease, reducing tumor burden, preventing metastasis of neoplasms and preventing recurrences of tumor/neoplastic growths in mammals.

The amount of the prodrug administered will depend upon the parent molecule included therein. Generally, the amount of prodrug used in the treatment methods is that amount which effectively achieves the desired therapeutic result in mammals. Naturally, the dosages of the various prodrug compounds will vary somewhat depending upon the parent compound, rate of in vivo hydrolysis, molecular weight of the polymer, etc. In general, however, prodrug taxanes are administered in amounts ranging from about 5 to about 500 mg/m^2 per day, based on the amount of the taxane moiety. Camptothecin prodrugs are also administered in amounts ranging from about 5 to about 500 mg/m^2 per day. The range set forth above is illustrative and those skilled in the art will determine the optimal dosing of the prodrug selected based on clinical experience and the treatment indication. Actual dosages will be apparent to the artisan without undue experimentation.

The prodrugs of the present invention can be included in one or more suitable pharmaceutical compositions for administration to mammals. The pharmaceutical compositions may be in the form of a solution, suspension, tablet, capsule or the like, prepared according to methods well known in the art. It is also contemplated that administration of such compositions may be by the oral and/or parenteral routes depending upon the needs of the artisan. A solution and/or suspension of the composition may be utilized, for example, as a carrier vehicle for injection or infiltration of the composition by any art known methods, e.g., by intravenous, intramuscular, subdermal injection and the like.

Such administration may also be by infusion into a body space or cavity, as well as by inhalation and/or intranasal routes. In preferred aspects of the invention, however, the prodrugs are parenterally administered to mammals in need thereof.

I. EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention. The underlined and bold-faced numbers recited in the Examples correspond to those shown in Figures 1-5.

General. All reactions were run under an atmosphere of dry nitrogen or argon. Commercial reagents were used without further purification. All PEG compounds were dried under vacuum or by azeotropic distillation (toluene) prior to use. ^{1}H spectra were obtained with a JEOL FT NMR System JNM GSX-270 instrument using deuteriochloroform as solvent unless specified. ^{13}C NMR spectra were obtained at 67.80 MHz on the JNM GSX-270. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) and coupling constants (J values) are given in Hertz (Hz). All PEG conjugated compounds were dissolved (~15 mg/mL) in sterile saline (0.9%) for injection prior to *in vivo* drug treatments and were given as their ara-C equivalents (absolute amount of ara-C given).

HPLC Method. Analytical HPLC's were performed using a C8 reversed phase column (Beckman, ultrasphere) under isocratic conditions with an 80:20 mixture (v/v) of methanol-water as mobile phase. Peak elutions were monitored at 254 nm using a UV detector. To detect the presence of any free PEG and also to confirm the presence of PEGYLATED product, an evaporative light scattering detector (ELSD), Model PL-EMD 950 (Polymer Laboratories), was employed. Based on ELSD and UV analysis, all the final PEGylated products were free of native drug and were ≥ 95% pure by HPLC.

Analysis of Ara-C Content in PEG Derivatives. For the determination of the ara-C content in PEG derivatives, *N*⁴-acetylcytidine was used as a model. The UV absorbance of *N*⁴-acetylcytidine in H_2O was determined at 257 nm for six different concentrations ranging from 0.01 μmol/mL to 0.05 μmol/mL. From the standard plot of absorbance vs. concentration, the absorption coefficient, ε, of *N*⁴-acetylcytidine was calculated to be 36.4 (O.D. at 257 nm for 1 mg/mL with 1.0 cm light path). PEGylated ara-C derivatives were

dissolved in H_2O at an approximate concentration of 0.015 μmol/mL (based on a MW of 40 kDa) and the UV absorbance of these compounds at 257 nm was determined. Using this value and employing the absorptive coefficient, ε, obtained from the above, the concentration of ara-C in the sample was determined. Dividing this value by the sample concentration provided the percentage of ara-C in the sample.

Analysis of Melphalan Content in PEG Derivatives. For the determination of the melphalan content in PEG derivatives, melphalan was used as a standard. The UV absorbance of melphalan in DMF-H_2O (9:1, v/v) was determined at 264 nm for five different concentrations ranging from 0.02 μmol/mL to 0.06 μmol/mL. From the standard plot of absorbance vs. concentration, the absorption coefficient, ε, of melphalan was calculated to be 54.6 (O.D. at 264 nm for 1 mg/mL with 1.0 cm light path). PEGYLATED melphalan derivatives were dissolved in DMF-H_2O (9:1, v/v) at an approximate concentration of 0.013 μmol/mL (based on a MW of 40 kDa) and the UV absorbance of these compounds at 264 nm was determined. Using this value and employing the absorption coefficient, ε, obtained from the above, the concentration of melphalan in the sample was determined. Dividing this value by the sample concentration provided the percentage of melphalan in the sample.

Abbreviations. DCM (dichloromethane), DMAP (4-(dimethylamino)pyridine), EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), HOBT (1-hydroxybenzotriazole), IPA (2-propanol), NMM (*N*-methylmorpholine), TFA (trifluoroacetic acid).

Example 1.

Compound 3a. A mixture of ara-C (1, 1.73 g, 7.12 mmol), 2a (700 mg, 1.78 mmol), HOBT (0.96 g, 7.12 mmol), and EDC-HCl (2.73 g, 14.25 mmol) in anhydrous pyridine (50 mL) was stirred at room temperature for 2 h, the temperature raised to 40 °C and the reaction continued overnight. The solvent was removed, methylene chloride (50 mL) was used to dissolve the mixture followed by washing with water (3 × 30 mL) and then with 0.1 N HCl (2 × 30 mL). The organic layer was dried over anhydrous $MgSO_4$, and the solvent removed *in vacuo* to give the crude product which was purified by silica gel column chromatography (5 to 10% MeOH in DCM) to give 638.8 mg (52%) of 3a as a white solid: ^1H NMR δ 1.42, 1.55, 2.17, 2.26, 2.46, 2.79, 3.84, 3.91, 4.14, 4.33, 4.53, 5.49, 6.07, 6.17, 6.52, 6.76, 7.31, 7.67, 8.16, 8.62; ^{13}C NMR δ 17.77, 20.11, 25.36, 28.32, 31.51, 31.96, 39.57, 50.18, 50.45, 61.88, 74.50, 80.15, 85.90, 88.58, 96.25, 122.51,

132.82, 133.34, 136.73, 138.22, 146.57, 149.90, 155.65, 155.96, 162.08, 171.89, 174.06.

Example 2.

Compound 3b. Compound 1 was coupled with 2b using a similar condition as in Example 1 to produce 3b in 54% yield: ^{13}C NMR δ 17.23, 17.92, 18.33, 25.49, 28.32, 31.51, 31.58, 31.99, 32.46, 39.52, 40.09, 50.08, 50.22, 61.72, 74.50, 74.94, 80.11, 80.15, 85.45, 85.90, 88.01, 88.58, 96.25, 122.51, 128.77, 129.03, 129.16, 131.68, 132.82, 136.24, 136.73, 138.22, 146.05, 146.57, 149.90, 155.65, 155.96, 171.85, 171.89, 174.06.

Example 3.

Compound 4a. Compound 3a (636.8 mg, 1.03 mmol) was stirred in anhydrous DCM (6 mL) and TFA (4 mL) at room temperature for 2 h. Ethyl ether was added to the solution to precipitate the crude product which was filtered and washed with ether to give 4a as a white solid (534.5 mg, 82%): ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 3H, $(CH_3)_2CH$) 1.55 (s, 3H, $(CH_3)_2CH$), 1.62 (d, 1 H, J = 8.1 Hz, $(CH_3)_2CH$), 2.22 (s, 3H, CH_3Ar), 2.57 (s, 3H, CH_3Ar), 2.97 (s, 2H, $CH_2C(=O)$), 3.41-4.27 (m, 5 H, sm-C's H-2'-H5'), 6.09 (d, 1H, J = 5.4, sm-C's H-1'), 6.67 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.12 (d, J = 5.4, H-6), 8.05 (d, J = 8.1, H-5), 8.67 (bs, 1H, TFA); ^{13}C NMR (DMSO-d_6) δ 15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53, 49.02, 61.02, 64.94, 74.64, 76.14, 85.74, 86.95, 94.32, 122.32, 132.41, 134.08, 135.67, 138.09, 146.71, 149.20, 154.50, 158.21, 158.72, 162.02, 169.68, 171.87.

Example 4.

Compound 4b. Compound 3b was subjected to the same condition as in Example 3 to give 4b in 82% yield: ^{1}H NMR (DMSO-d_6) δ 1.52 (s, 3H, $(CH_3)_2CH$) 1.55 (s, 3H, $(CH_3)_2CH$), 1.62 (d, 1 H, J = 8.1 Hz, $(CH_3)_2CH$), 2.22 (s, 3H, CH_3Ar), 2.57 (s, 3H, CH_3Ar), 2.97 (s, 2H, $CH_2C(=O)$), 3.41-4.27 (m, 5 H, sm-C's H-2'-H5'), 6.09 (d, 1H, J = 5.4, sm-C's H-1'), 6.67 (s, 1H, Ar-H), 6.90 (s, 1H, Ar-H), 7.12 (d, J = 5.4, H-6), 8.05 (d, J = 8.1, H-5), 8.67 (bs, 1H, TFA); ^{13}C NMR (DMSO-d_6) δ 15.45, 19.67, 24.97, 31.05, 31.23, 38.56, 40.41, 48.53, 49.02, 61.02, 64.94, 74.64, 76.14, 85.74, 86.95, 94.32, 122.32, 132.41, 134.08, 135.67, 138.09, 146.71, 149.20, 154.50, 158.21, 158.72, 162.02, 169.68, 171.87.

Example 5.

Compound 6a. A mixture of PEG-aspartic acid (mw. 40,000, 5.3 g, 0.074 mmol), 4a (385.6 mg, 0.74 mmol), NMM (240 mg, 2.38 mmol), HOBT (120.5 mg, 0.89 mmol), and

EDC·HCl (228.4 mg, 1.19 mmol) in anhydrous DCM (50 mL) was stirred at 0 °C for 30 minutes. The reaction was allowed to warm to room temperature and continued for 3 days and filtered. The filtrate was concentrated *in vacuo* and the residue recrystallized from IPA to give 2.7 g (90%) of product. The amount of ara-C in the product measured by UV assay was 2.11 wt%: ^{13}C NMR δ 14.40, 19.22, 24.86, 31.17, 38.26, 38.90, 47.94, 48.67, 49.66, 60.17, 61.12, 61.90, 67.86-70.87 (PEG), 71.70, 74.50, 85.01, 87.53, 95.28, 121.39, 131.18, 132.68, 133.19, 134.77, 137.70, 145.26, 158.93, 155.23, 160.12, 161.56, 168.39, 170.72, 170.92, 171.27, 171.34.

Example 6.

Compound 6b. Compound 4b was subjected to the same condition as in Example 5 to give 6b in 88% yield. The amount of ara-C in the product measured by UV assay was 1.68 wt%: ^{13}C NMR δ 15.12, 16.22, 24.52, 24.73, 29.55, 30.55, 31.15, 38.04, 38.59, 47.66, 49.16, 49.93, 50.18, 60.93, 61.12, 62.90, 69.44-71.59 (PEG), 71.70, 74.50, 84.78, 84.90, 87.53, 94.85, 127.60, 130.20, 135.51, 136.10, 141.70, 145.15, 147.50, 155.00, 161.20, 169.47, 170.62, 170.92, 171.27.

Example 7.

Compound 9. PEG diol (7, 55 g, 1.38 mmol) was azeotroped in toluene over a 2 hour period followed by removal of 200 mL of solvent by rotary evaporation. The solution was cooled to ~30 °C and triphosgene (0.544 g, 1.83 mmol) was added as solid followed by anhydrous pyridine (0.434 g, 5.49 mmol), and the reaction mixture stirred at 50 °C for 1 hour. *N*-hydroxyphthalimide (8, 1.12 g, 6.88 mmol) and anhydrous pyridine (0.54 g, 6.88 mmol) were added to the chloroformate mixture and the reaction stirred for a further 2 hours at 50 °C then for 12 hours at room temperature. The reaction mixture was filtered through filter paper and the solvent removed *in vacuo* and the product crystallized from methylene chloride-ethyl ether (1100 mL, 8:2, v/v) to give the product (50.9 g, 92%): ^{13}C NMR δ 123.62, 128.10, 134.55, 152.00, 160.00.

Example 8.

PEG-cme-Asp-O-*t*-Bu (11). Compound 9 (mw. 40,000, 20 g, 0.459 mmol) and aspartic acid di *t*-butyl ester HCl (10, 1.0 g, 3.55 mmol) were dissolved in anhydrous DCM, followed by addition of DMAP (0.433 g, 3.55 mmol). The solution was refluxed overnight followed by precipitation by addition of ethyl ether (1 L). The solid was isolated by filtration and recrystallized from IPA (1 L) twice. The filter cake was washed

with IPA (200 mL) and ether (200 mL) to give 15.6 g (78%) of product after drying at 45 °C *in vacuo*: ^{13}C NMR δ 27.837 ($CH_3CO_2C(CH_3)_3$), 27.991 ($CHCO_2C(CH_3)_3$), 37.752 ($CHCH_2CO_2$), 50.800 (NHCH), 64.212 ($OCH_2CH_2OC(=O)NH$), 81.333 ($CH_2CO_2C(CH_3)_3$), 82.007 ($CHCO_2C(CH_3)_3$), 155.924 ($OCH_2CH_2OC(=O)NH$), 169.674 ($CH_2CO_2C(CH_3)_3$), 169.969 ($CHCO_2C(CH_3)_3$).

Example 9.

PEG-cmc-Asp-OH (12). Compound 11 (15 g, 0.375 mmol) was dissolved in DCM (150 mL) followed by the addition of TFA (75 mL). The solution was stirred at room temperature for 2 hours and hexane (500 mL) added to precipitate the solid. The solid was triturated with hexane to remove TFA followed by recrystallization from chilled DCM-ether. The recrystallized solid was redissolved in DCM (150 mL) and washed with water (150 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, concentrated *in vacuo*, and precipitated with ether to give 12.4 g (83%) of product: ^{13}C NMR δ 36.441 ($CHCH_2CO_2$), 50.177 (NHCH), 64.390 ($OCH_2CH_2OC(=O)NH$), 81.333 ($CH_2CO_2C(CH_3)_3$), 82.007 ($CHCO_2C(CH_3)_3$), 156.172 ($OCH_2CH_2OC(=O)NH$), 171.944 ($CH_2CO_2C(CH_3)_3$), 172.211 ($CHCO_2C(CH_3)_3$).

Example 10.

Boc-Asp-Asp-OMe (15). EDC·HCl (2.47 g, 12.86 mmol) was added to a mixture of BocNH-aspartic acid (13, 1 g, 4.29 mmol), aspartic acid dimethyl ester·HCl (14, 1.86 g, 9.43 mmol), and DMAP (2.47 g, 12.86 mmol) in anhydrous DCM (30 mL) and DMF (2 mL) at 0 °C. The mixture was allowed to warm up to room temperature overnight. The mixture was washed with 1N HCl three times and the organic layer was dried over anhydrous $MgSO_4$, followed by removal of the solvent *in vacuo* to give the product (2.0 g, 90%): ^{1}H NMR δ 1.45 (s, 9H), 2.62-3.02 (m, 6H, 3 × CH), 3.70 (s, 6H, 2 × OCH_3), 3.74 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.50 (bs, 1H, CH), 4.85 (m, 2H, 2 × CH), 6.05 (d, J = 6.95 Hz, 1H, NH), 6.98 (d, J = 8.05 Hz, 1H, NH), 7.57 (d, J = 7.69 Hz, 1H, NH).

Example 11.

Asp-Asp-OMe (16). Compound 15 (2.0 g, 3.35 mmol) was dissolved in DCM (30 mL) and TFA (15 mL) and the solution was stirred for 2 h at room temperature. The solvent was removed *in vacuo* and the residue was recrystallized twice with DCM-ether to give the product (1.74 g, 87%) as a white solid: ^{13}C NMR δ 35.52, 48.76, 50.12, 51.90, 51.96, 52.65, 114.59, 118.69, 168.43, 170.02, 170.92, 171.17, 171.40, 171.48.

Example 12.

PEG-cmc-Asp-Asp-OMe (17). DMAP (4.5 g, 36.86 mmol) was added to a solution of 9 (mw. 40,000, 74 g, 1.84 mmol) and 16 (9.83 g, 18.43 mmol) in 700mL of anhydrous chloroform. The reaction mixture was refluxed for 24 hours under nitrogen. The reaction was cooled to room temperature and concentrated to ½ volume. Crude product was precipitated with 2.5 L of ether, filtered and recrystallized from 5.5 L of IPA (65°C). The product was filtered and washed twice with fresh IPA, twice with fresh ether, and dried overnight at 40 °C to yield 59.0g (84%) of 17: ^{13}C NMR δ 35.344, 36.931, 48.082, 48.208, 50.835, 51.509, 52.239, 61.045, 63.953, 68.854-72.056, 155.538, 170.102, 170.369, 170.453, 170.734.

Example 13. .

PEG-cmc-Asp-Asp-OH (18). Compound 17 (51 g, 1.26 mmol) and LiOH-H_2O (0.8 g, 18.9 mmol) were dissolved in 300 mL of water and the solution stirred overnight at room temperature. The pH of the solution was adjusted to 2.5 by the addition of 1N HCl. The solution was extracted with DCM (3 × 600 mL), the organic layers combined, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from DCM-ether to give the product which was collected by filtration and dried at 40 °C overnight to yield 38 g (54%) of the octa-acid: ^{13}C NMR (D_2O) δ 38.384, 39.704, 51.951, 54.465, 62.934, 67.165, 71.445-74.381 (PEG), 159.772, 173.831, 174.940, 176.359, 176.696.

Example 14.

Mel-OMe (20). Melphalan (19, 1.00 g, 3.28mmol) was suspended in 2,2 dimethoxypropane (65.59 mL, 533.49 mmol). To the suspension was added aqueous HCl (36 %, 3.28 mL) and absolute methanol (4 mL). The mixture was warmed to mild reflux with vigorous stirring until solution started to turn slightly brown, followed by stirring at room temperature for 18 hours. The reaction mixture was concentrated *in vacuo* and the crude product precipitated from the residue with ether. The solid was filtered, washed with ether, and purified by silica gel column chromatography ($CHCl_3$: MeOH = 9:1, v/v) to yield the desired product (0.47g, 45%): ^{13}C NMR δ 39.751, 40.340, 51.912, 53.435, 55.803, 112.124, 126.076, 130.620, 145.033, 175.754.

Example 15.

Boc-TML1β-Mel-OMe (22). EDC (0.52 g, 2.70 mmol) and DMAP (0.988 g, 8.10 mmol) were added to a mixture of 21 (0.531 g, 1.35 mmol) and 20 (0.863 g, 2.70 mmol) in

anhydrous DCM (15 mL) and anhydrous DMF (5 mL) at 0 °C in an ice bath.. The reaction mixture was stirred at room temperature overnight under nitrogen then concentrated *in vacuo*. The residue was redissolved in DCM (75 mL) and washed three times with 25mL 1N HCl. The organic layer was dried over anhydrous magnesium sulfate, concentrated, and purified by silica gel column chromatography (ethyl acetate:hexane = 7:3, v/v) to yield the desired product (0.757 g, 80.8 %): ^{13}C NMR δ 20.120, 25.306, 28.294, 31.768, 35.427, 35.947, 36.669, 39.505, 40.311, 49.324, 51.959, 52.234, 53.453, 79.467, 112.095, 123.374, 125.160, 130.439, 132.856, 133.427, 136.666, 138.697, 145.091, 149.841, 156.081, 170.888, 172.298.

Example 16.

TML1β-Mel-OMe TFA Salt (23). Compound 22 (0.757 g, 1.09 mmol) was stirred in DCM (5mL) and TFA (2.5 mL) at room temperature for 2 hours. The reaction solution was concentrated, redissolved in minimal DCM, and precipitated with ether. The product was collected by filtration to yield the desired product (0.222g, 35.9 %): ^{13}C NMR (CDCl$_3$ + CD$_3$OD) δ 20.026, 25.146, 31.738, 31.892, 35.271, 36.219, 39.163, 40.340, 49.006, 52.219, 53.396, 112.073, 123.260, 124.756, 130.377, 133.026, 133.180, 136.815, 138.595, 145.110, 149.283, 171.069, 171.619, 172.630.

Example 17.

PEG-cmc-TML1β-Mel-OMe (24). A mixture of PEG-cmc-Asp-Asp-OH (12, 1.6g, 0.0391mmol), 23 (0.277g, 0.391mmol), EDC (0.076g, 0.391mmol), and DMAP (0.155g, 1.269mmol) in anhydrous DCM (23 mL) and anhydrous DMF (6 mL) was stirred overnight at room temperature under nitrogen. The solution was concentrated *in vacuo* and the residue recrystallized from 130mL IPA to yield the product (1.543g, 92.5 %). The amount of melphalan in the product measured by UV assay was 2.86% wt/wt: ^{13}C NMR δ 19.642, 24.788, 31.175, 34.350, 35.975, 38.817, 39.905, 48.558, 51.553, 52.803, 60.897, 62.331, 65.145-72.878 (PEG), 111.394, 122.761, 124.425, 129.698, 132.105, 132.878, 135.804, 137.737, 144.316, 149.065, 160.432, 170.608, 171.598.

Example 18.

Boc-TML1β-AraC (25). A solution of Ara-C (1, 9.88 g, 40.66 mmol) in anhydrous pyridine (85 mL) was added to a mixture of 21 (4.0 g, 10.17 mmol), HOBT (5.49 g, 40.66 mmol), EDC (15.61 g, 81.32 mmol), and NMM (8.93mL, 8.21g, 81.32mmol, 8eq) in anhydrous pyridine (200 mL). The reaction mixture was stirred for 48 hours at 40 °C

under nitrogen, followed by concentration *in vacuo*. The residue was redissolved in DCM (300 mL), washed three times with water (100 mL) and twice with 0.1N HCl (100 mL). The organic layer was dried over magnesium sulfate, concentrated, and purified by silica gel column chromatography ($CHCl_3$ - MEOH = 9:1, v/v) to yield the desired product (3.26 g, 52 %): ^{13}C NMR δ 20.315, 25.560, 28.522, 31.660, 35.520, 36.200, 39.221, 50.239, 61.719, 75.171, 76.698, 79.635, 85.341, 88.052, 96.435, 122.894, 132.519, 133.190, 136.186, 158.007, 146.222, 149.109, 155.906, 162.191, 171.733.

Example 19.

TML1β-AraC TFA salt (26). Compound 25 (3 g, 4.85 mmol) was dissolved in DCM (15 mL) followed by addition of TFA (7.5 mL) at 0 °C. Reaction mixture was stirred at 0 °C for 1.2 hours and concentrated *in vacuo* in a cool water bath. Residue was precipitated with DCM-ether to yield the desire product (2.37 g, 77 %): ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 20.0, 25.3, 31.5, 31.7, 35.0, 38.9, 50.2, 60.9, 75.1, 75.8, 85.7, 88.1, 94.9, 109.7, 113.5, 117.3, 121.1, 122.5, 132.6, 136.4, 138.4, 148.7, 149.5, 150.1, 159.2, 159.6, 160.1, 160.6, 161.1, 170.6, 172.7

Example 20.

PEG-exc-Asp-Asp-TML1β-AraC, octamer (27). Compounds 26 and 18 were subjected to the same condition as in Example18 to prepare 27.

Example 21.

In vitro and in vivo data for compounds 6a and 6b.

In this Example, in vivo and in vitro data are presented and compared to unmodified Ara-C.

In Vivo

Athymic nude mice were implanted subcutaneous with a 4-5 mm^3 tissue fragment of LX-1 collected from donor mice. The tumor inocar site was observed twice weekly and measured once palpable. The tumor volume for each mouse was determined by measuring two dimensions with calipers and calculated using the formula: tumor volume = (length x width^2)/2. When tumors reached the average volume of 90 mm^3, the mice were divided into their experimental groups which consisted of unmodified Ara-C and PEG-Ara-C compounds. The mice were sorted to evenly distribute tumor size, grouped into 4 to 6 mice/group, and ear punched for permanent identification. Drugs were administered intravenously q3d x 4 (Day 1, 4, 7 and 10) via the tail vein at an approximate rate of 0.5

mL per minute. Compounds were given both at an equal molar basis (absolute amount of active) of 20 mg/kg and at close their respective MTD (Ara-C, 100 mg/kg/dose (toxicity); 6a and 6b, 40 mg/kg/dose (volume). Mouse weight and tumor size were measured at the beginning of study and twice weekly through week 4. Drug effectiveness was determined by comparing tumor growth in treated versus untreated (no vehicle) control mice. Five types of endpoints were used as the basis for comparison: (a) mean tumor volumes at Day 28; (b) mean percent change in individual tumor volumes from initial; (c) percent difference in tumor volume (%T/C), measured when the control group's median tumor volume reached approximately 800 - 1100 mm^3 (exponential growth phase); (d) percent difference in tumor volume (%T/C) at Day 21 (~2000 mm^3) and (e) the number of tumor regression (smaller tumor volume on Day 28 compared to Day 1) per group.

Results

Compound 6b demonstrated better antitumor activity than native Ara-C at only 20% of the active parent compound's dose. Compound 6a also demonstrated significant efficacy. Although the %T/C was about twice of that which was recorded for 6b, it nonetheless compared very favorably against native Ara-C, especially considering that the inventive compound was given at only 20% of the active parent compound's dose.

Compound	$t_{1/2}$ (h)[a] Rat Plasma	IC_{50} (nM)[a] P388/O	LX-1 %T/C[b]
Ara-C	-	10	74.0 (100 mg / kg)
Compound 6a	2.1	123	122 (20 mg / kg)
Compound 6b	53	958	59.3 (20 mg / kg)

[a] All experiments were done at 37 °C in duplicate and $t_{1/2}$ was measured by the disappearance of PEG derivatives. Standard deviation of measurements = ±10 %.
[b] Mean baseline tumor volume was 1000 mm^3.

IN VITRO BIOASSAY

A series of in vitro assays were conducted to determine the IC_{50} for unmodified Ara-C and compound 10 using the P388/O (murine lymphoid neoplasm, Southern Research Institute) cell line. The P388/0 cells were grown in RPMI 1640 medium (Whittaker Bioproducts, Walkersville, Maryland) + 10% FBS (Hyclone Inc., Logan UT). Bioassays were performed in their respective media containing antibiotics and fungizone.

Ara-C was dissolved in DMSO and diluted to the appropriate concentration in culture media. The PEG-Ara-C compounds were dissolved in water and diluted to the appropriate concentrations in culture media.

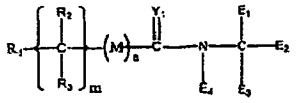
The assays were performed in duplicate in 96-well microtiter cell culture plates. Two fold serial dilution of the compounds were done in the microtiter plates. Cells were detached by incubating with 0.1% Trypsin/Versene at 37°. Trypsin was inactivated by adding the appropriate media for each cell line containing 10% FBS. To each well of the microtiter plates, 10,000 cells were added. After three days, cell growth was measured by addition of a metabolic indicator dye, Alamar Blue, according to the manufacturer's protocol. The IC_{50} value for the test compounds and reference compound are provided above in the Table.

While there have been described what are presently believed to be the preferred embodiments of the invention, those skilled in the art will realize that changes and modifications may be made without departing from the spirit of the invention. It is intended to claim all such changes and modifications as fall within the true scope of the invention.

WHAT IS CLAIMED IS:

1. A compound comprising the formula:

(I)



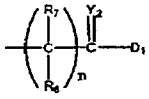
wherein:

R_1 is a polymeric residue;

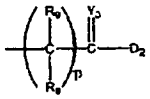
Y_1 is O, S or NR_4;

M is O, S or NR_5;

E_1 is



E_{2-4} are independently H, E_1 or



(a) is zero or one;

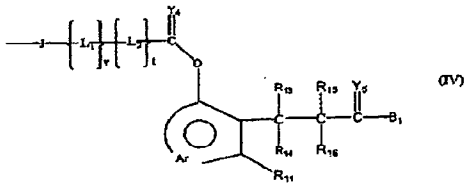
(m) is zero or a positive integer;

(n) and (p) are independently 0 or a positive integer;

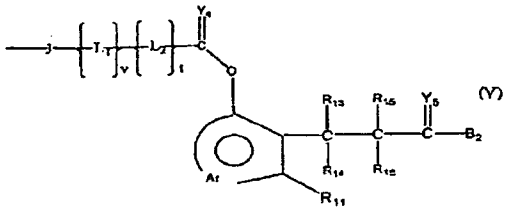
$Y_{2,3}$ are independently O, S or NR_{10};

R_{2-9} are independently selected from the group consisting of hydrogen, C_{1-4} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

D_1 and D_2 are independently OH.



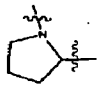
(IV)



(V)

or a terminal branching group;

wherein (v) and (t) are independently 0 or a positive integer up to about 6;

J is NR_{17} or 

L_1 and L_2 are independently selected bifunctional linkers;

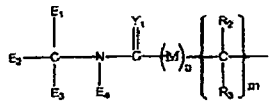
Y_{4-7} are independently selected from the group consisting of O, S and NR_{14};

R_{11-14} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

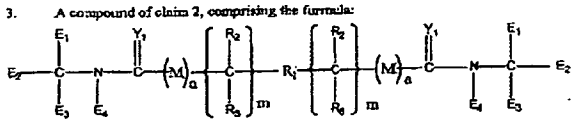
Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group;

B_1 and B_2 are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing moieties or amine-containing moieties.

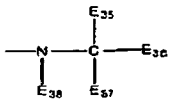
2. The compound of claim 1, wherein R_1 further comprises a capping group A, selected from the group consisting of hydrogen, NH_2, OH, CO_2H, C_{1-6} moieties and



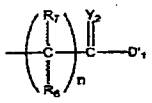
3. A compound of claim 2, comprising the formula:

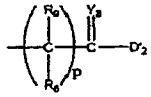


4. The compound of claim 1, wherein said terminal branching group comprises the formula:



wherein

E_{35} is 

E_{36-38} are independently H, R_{10} or 

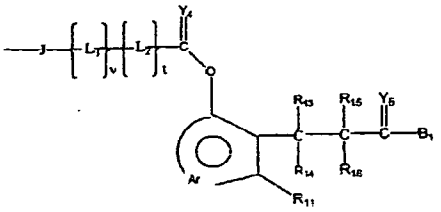
(n) and (p) are independently 0 or a positive integer;

Y_{2-3} are independently O, S or NR_{10};

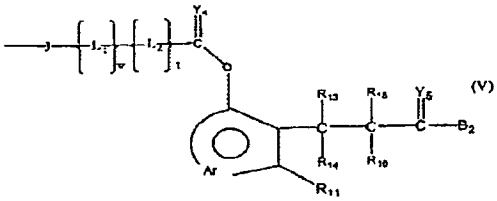
R_{6-10} are independently selected from the group consisting of hydrogen, C_{1-4} alkyls, C_{3-12} branched alkyls, C_{3-6} cycloalkyls, C_{1-6} substituted alkyls, C_{2-6} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{2-6} heteroalkyls, substituted C_{1-6} hetero-

alkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

D'_1 and D'_2 are independently OH,

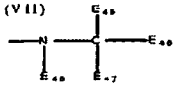


(IV)



(V)

or



(VII)

wherein (v) and (t) are independently 0 or a positive integer up to about 6;

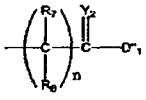
L_1 and L_2 are independently selected bifunctional linkers;

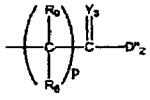
Y_{1-7} are independently selected from the group consisting of O, S and NR_{16};

R_{1-16} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-10} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group;

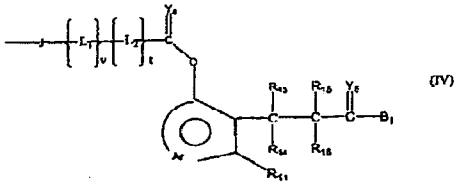
B_1 and B_2 are independently selected from the group consisting of leaving groups, OH, residues of hydroxyl-containing moieties or amine-containing moieties;

E_{e1} is 

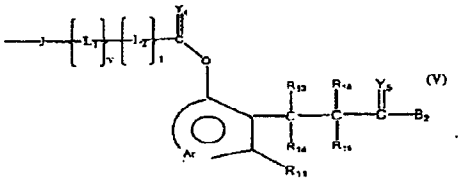
E_{e2-n} are independently H, E_{e1} or 

wherein

D''_1 and D''_2 are independently OH,


(IV)

or


(V)

5. The compound of claim 3, Y_1 is O.

6. The compound of claim 1, wherein R_1 comprises a polyalkylene oxide residue.

7. The compound of claim 6, wherein R_1 comprises a polyethylene glycol residue.

8. The compound of claim 3, wherein R_1 comprises a polyethylene glycol residue.

9. The compound of claim 6, wherein R_1 is selected from the group consisting of
$-C(=Y_4)-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-C(=Y_4)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-C(=Y_4)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-NR_{26}-(CH_2)_f-O-(CH_2CH_2O)_x-A$,
$-C(=Y_4)-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-C(=Y_4)-$,
$-C(=Y_4)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-Y_7-C(=Y_4)-$,
$-C(=Y_4)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{23}-C(=Y_4)-$,
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-O-(CR_{24}R_{25})_e-$, and
$-NR_{26}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{26}-$

wherein: Y_4 and Y_7 are independently O, S or NR_{23};

x is the degree of polymerization;

R_{23}, R_{24} and R_{25} are independently selected from among H, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

e and f are independently zero, one or two; and

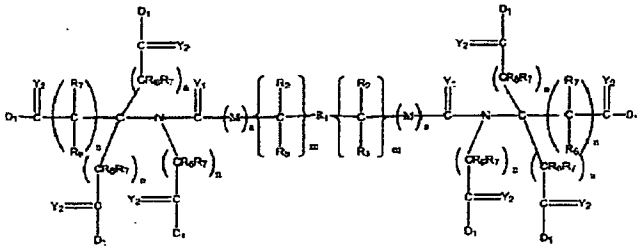
A is a capping group.

10. The compound of claim 9, wherein R_1 comprises $-O-(CH_2CH_2O)_x$ and x is a positive integer so that the weight average molecular weight is at least about 20,000.

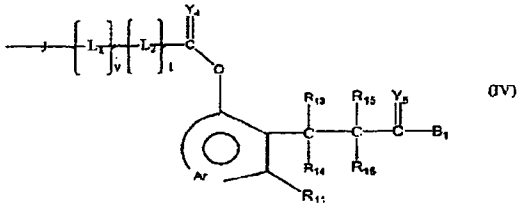
11. The compound of claim 3, wherein R_1 has a weight average molecular weight of from about 20,000 to about 100,000.

12. The compound of claim 3, wherein R_1 has a weight average molecular weight of from about 25,000 to about 60,000.

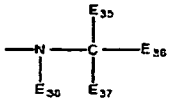
13. A compound of claim 3, comprising the formula



14. The compound of claim 13, wherein D_1 is



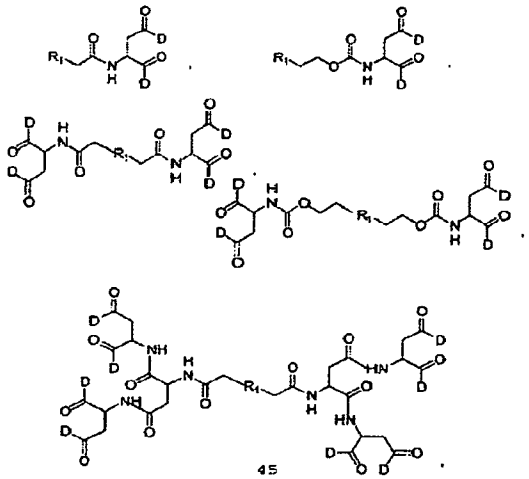
(IV)

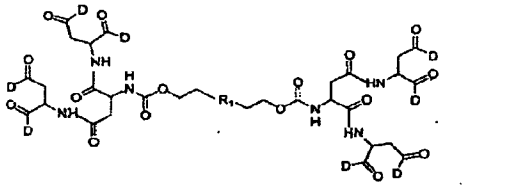
15. The compound of claim 13, wherein D_1 is 

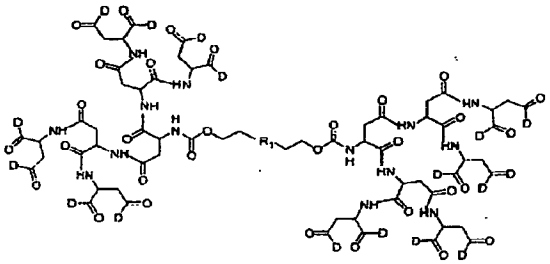
16. The compound of claim 1, wherein L_1 is $(CH_2CH_2O)_2$.

17. The compound of claim 1, wherein L_2 is selected from the group consisting of $-CH_2-$, $-CH(CH_3)-$, $-CH_2C(O)NHCH(CH_3)-$, $-(CH_2)_2-$, $-CH_2C(O)NHCH_2-$, $-(CH_2)_3NH-$, $-(CH_2)_3-NH-C(O)(CH_2)_2NH-$ and $-CH_2C(O)NHCH(CH_2CH(CH_3)_2)-$.

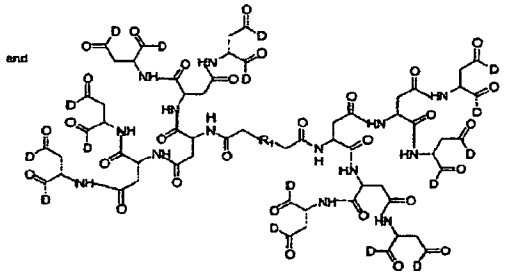
18. A compound of claim 1, selected from the group consisting of:





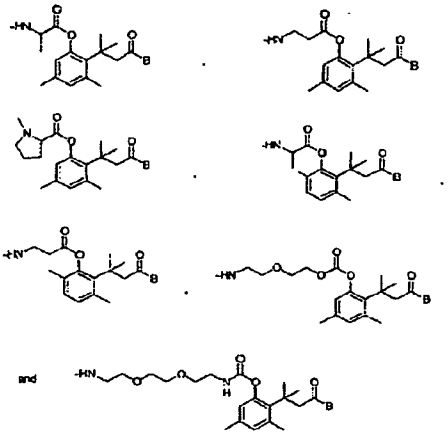


and



46

wherein R_1 is a PEG residue and D is selected from the group consisting of:



and

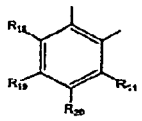
where B is a residue of an amine or a hydroxyl- containing drug.

19. A compound of claim 18, wherein B is a residue of a member of the group consisting of: daunorubicin, doxorubicin; *p*-aminoaniline mustard, melphalan, Ara-C (cytosine arabinoside), leucine-Ara-C, and gemcitabine

20. A method of treatment, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 1, wherein D, is a residue of a biologically active moiety.

21. A method of treatment, comprising administering to a mammal in need of such treatment an effective amount of a compound of claim 18.

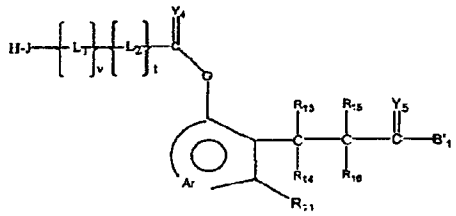
22. The compound of claim 1, wherein Ar comprises the formula:



wherein R_{11} and R_{13-20} are individually selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{1-6} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy.

23. The compound of claim 22, wherein R_{11} and R_{13-20} are each H or CH_3.

24. A method of preparing a polymer conjugate, comprising:
reacting a compound of the formula (VIII):

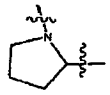


(VIII)

wherein

(v) and (t) are independently 0 or a positive integer up to about 6;

J is NR_{17} or



L_1 and L_2 are independently selected bifunctional linkers;

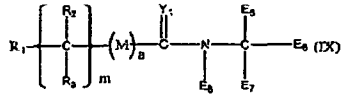
Y_{1-3} are independently selected from the group consisting of O, S and NR_{11};

R_{1-11} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

Ar is a moiety which when included in Formula (I) forms a multi-substituted aromatic hydrocarbon or a multi-substituted heterocyclic group; and

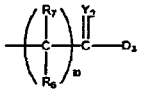
B', is a residue of a hydroxyl- or an amine-containing moiety;

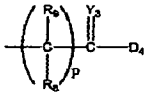
with a compound of the formula (IX):



(IX)

wherein

E_5 is 

E_{6-8} are independently H, E_5 or 

D_3 and D_4 are independently OH, a leaving group which is capable of reacting with an unprotected amine or hydroxyl or a terminal branching group;

R_1 is a polymeric residue;

Y_1 is O, S or NR_9;

M is O, S or NR_{10};

(a) is zero or one;

(m) is 0 or a positive integer;

(n) and (p) are independently 0 or a positive integer;

Y_{2-3} are independently O, S or NR_{11}; and

R_{1-20} are independently selected from the group consisting of hydrogen, C_{1-6} alkyls, C_{3-12} branched alkyls, C_{3-8} cycloalkyls, C_{1-6} substituted alkyls, C_{3-8} substituted cycloalkyls, aryls, substituted aryls, aralkyls, C_{1-6} heteroalkyls, substituted C_{1-6} heteroalkyls, C_{1-6} alkoxy, phenoxy and C_{1-6} heteroalkoxy;

under conditions sufficient to cause a polymeric conjugate to be formed.

Fig. 1

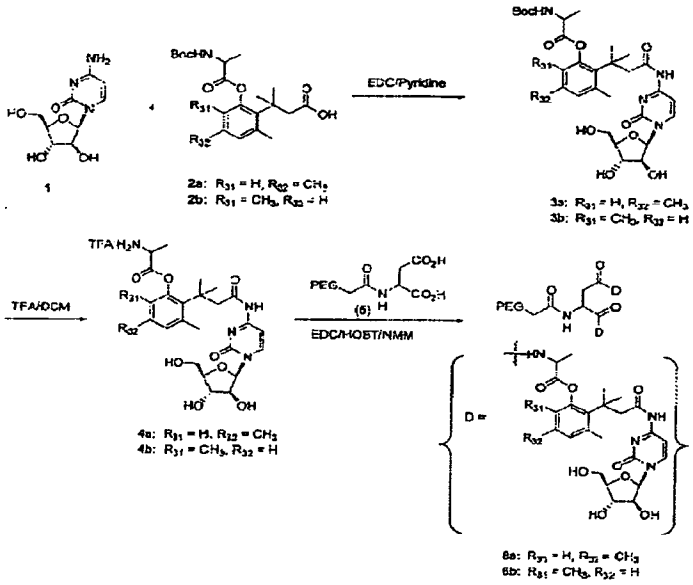

EDC/Pyridine
TFA/DCM
EDC/HOBT/NMM
2a: R31 = H, R32 = CH3
2b: R31 = CH3, R32 = H
3a: R31 = H, R32 = CH3
3b: R31 = CH3, R32 = H
4a: R31 = H, R32 = CH3
4b: R31 = CH3, R32 = H
6a: R31 = H, R32 = CH3
6b: R31 = CH3, R32 = H

Fig. 2

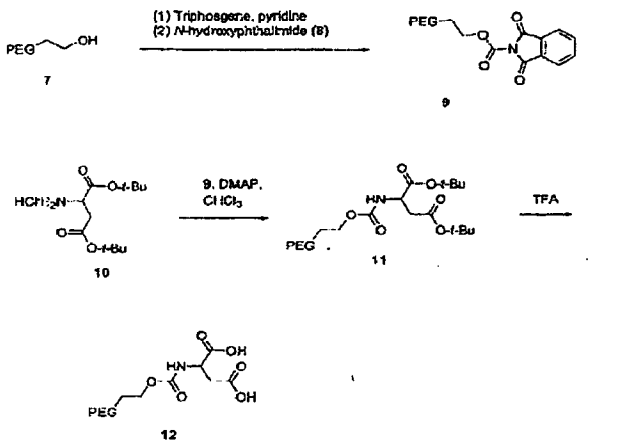

(1) Triphosgene, pyridine
(2) N-hydroxyphthalimide (8)
PEG
OH
7
9
HCl·H2N
O-t-Bu
10
9, DMAP,
CHCl3
HN
11
TFA
12

Fig. 3

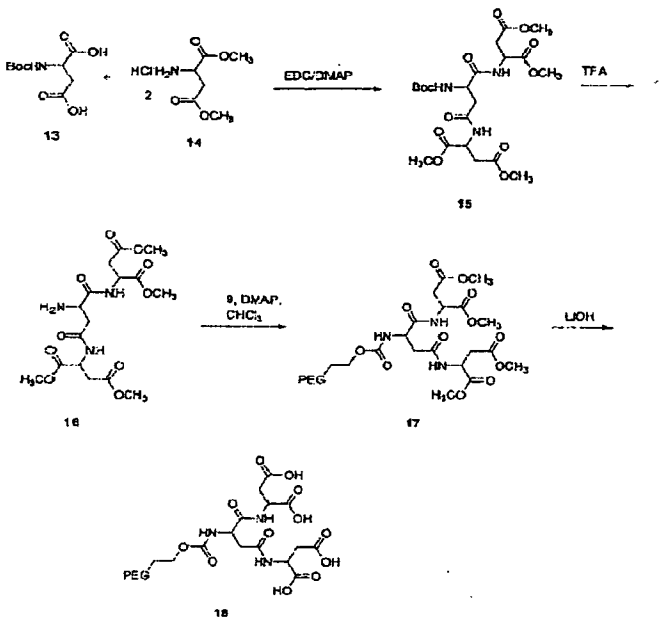


Fig. 4

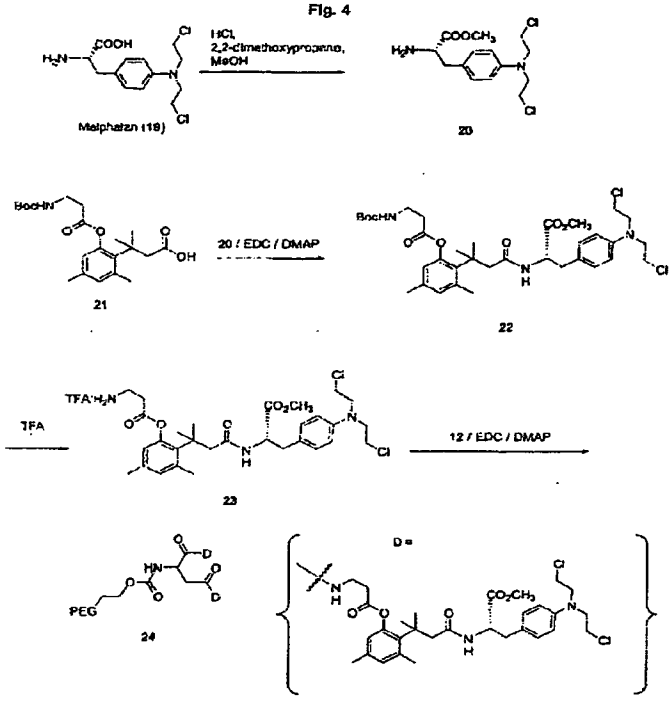
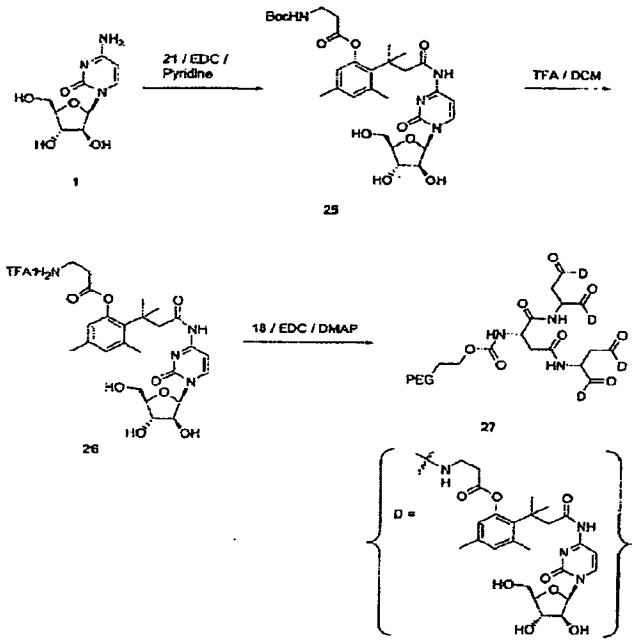

HCl
2,2-dimethoxypropane,
MeOH
Melphalan (19)
20
20 / EDC / DMAP
21
22
TFA
TFA·H2N
23
12 / EDC / DMAP
PEG
24
D =

Fig. 5



【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/01780

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 47/30, 39/385, 38/34
US CL : Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 568/332, 462, 463; 525/54.1; 560/160, 170, 200; 514/772.6, 513, 515, 615, 424/178.1, 193.1, 178.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS, STN, search terms: polymer?, conjugat?, branch?, camptothecin?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GREENWALD et al.; Drug delivery systems based on trimethyl lock lactonization: Poly(ethylene glycol) Prodrugs of amino-containing compounds; 2000, Chem Abstract 132: 227266	1-24
Y	GREENWALD et al; "Trialkyl-lock-facilitated polymeric prodrugs of amino-containing bioactive agents"; 1999; Chem Abstract 131; 92540	1-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
18 MAY 2002

Date of mailing of the international search report
18 JUN 2002

Name and mailing address of the ISA/US
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Box PCT
Washington, D.C. 20231
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Authorized officer
DUC TRUONG
Telephone No. (703) 308-0661

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/04783

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

[illegible]

Form PCT/ISA/210 (extra sheet) (July 1998)*

フロントページの続き

(81)指定国 AP(GH,GM,KE,LS,MW,MZ,SD,SL,SZ,TZ,UG,ZM,ZW),EA(AM,AZ,BY,KG,KZ,MD,RU,TJ,TM),EP(AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE,TR),OA(BF,BJ,CF,CG,CI,CM,GA,GN,GQ,GW,ML,MR,NE,SN,TD,TG),AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DZ,EC,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NO,NZ,OM,PH,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TN,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZM,ZW

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アメリカ合衆国 ０８８１２ ニュージャージー州，グリーン ブルック，グリーンブライアー ロード ９
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アメリカ合衆国 ０８８７３ ニュージャージー州，サマーセット，ヒッコリー ロード １１３

Ｆターム(参考) 4C076 AA95 CC27 CC42 EE59 FF31
4J005 AA02 BD05 BD06
4J031 CD13

【公報種別】特許法第１７条の２の規定による補正の掲載
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【発行日】平成17年12月22日(2005.12.22)

【公表番号】特表2004-530736(P2004-530736A)
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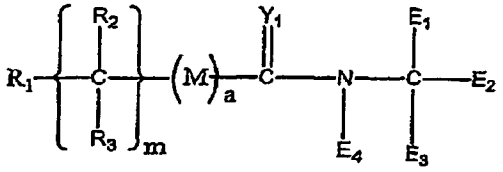
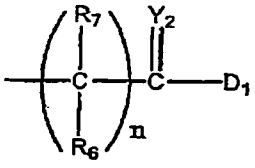
【年通号数】公開・登録公報2004-039
【出願番号】特願2002-565624(P2002-565624)
【国際特許分類第７版】
　Ｃ０８Ｇ　85/00
　Ａ６１Ｋ　47/48
　Ｃ０８Ｇ　65/329
【ＦＩ】
　Ｃ０８Ｇ　85/00
　Ａ６１Ｋ　47/48
　Ｃ０８Ｇ　65/329

【手続補正書】
【提出日】平成16年9月29日(2004.9.29)
【手続補正１】
【補正対象書類名】特許請求の範囲
【補正対象項目名】全文
【補正方法】変更
【補正の内容】
【特許請求の範囲】
【請求項１】
【式１】

(I)

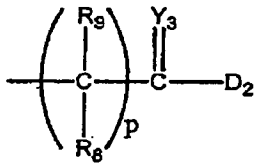


式中、
R_1は高分子残基であり；
Y_1はO、SまたはNR$_4$であり；
MはO、SまたはNR$_5$であり；
E_1は
【化２】



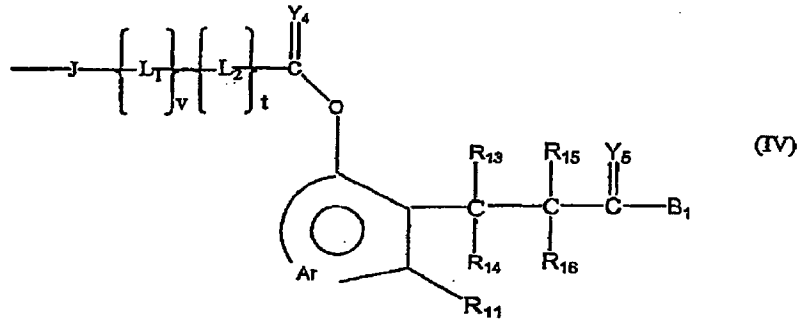
であり；
E_{2-4}は独立に、H、E_1または

【化３】

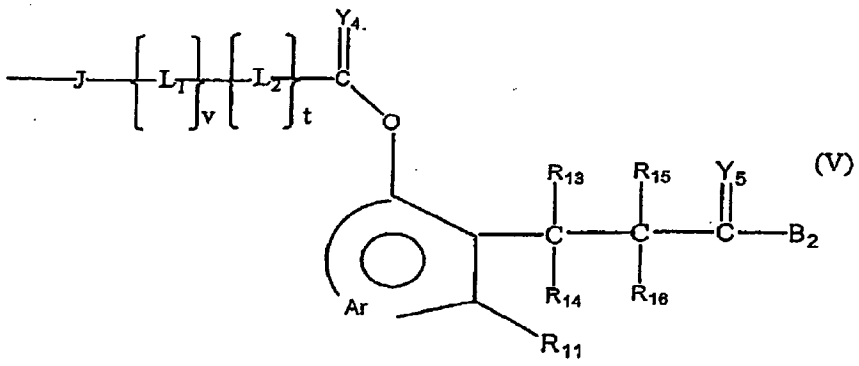


であり；
(a)は0または1であり；
(m)は0または正の整数であり；
(n)および(p)は独立に、0または正の整数であり；
Y_{2-3}は独立に、O、SまたはNR$_{10}$であり；
R_{2-10}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
D_1およびD_2は独立に、OH、

【化４】



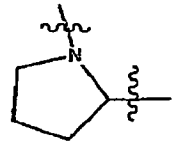
(IV)



(V)

(式中、
(v)および(t)は独立に、0または約6までの正の整数であり；
JはNR$_{12}$または

【化５】



であり；

L_1およびL_2は独立に選択された二官能性リンカーであり；

Y_{4-7}は独立に、O、SおよびNR_{14}からなる群から選択され；

R_{11-14}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；

Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり；

B_1およびB_2は独立に、脱離基、OH、ヒドロキシル基含有成分またはアミン基含有成分の残基からなる群から選択される）

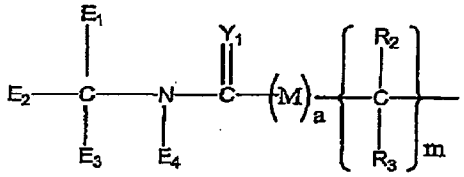
または末端分枝基である｝

で表される化合物。

【請求項２】

R_1が水素、NH_2、OH、CO_2H、C_{1-6}基および

【化６】

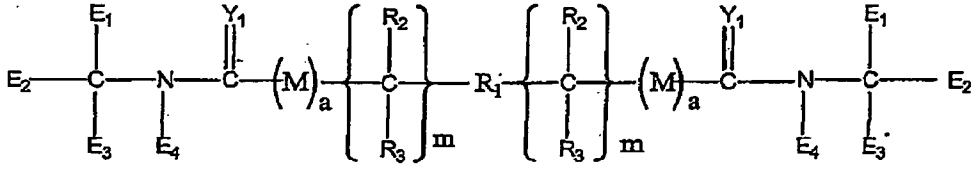


からなる群から選択されるキャッピング基Aをさらに含んでなる、請求項１に記載の化合物。

【請求項３】

式：

【化７】

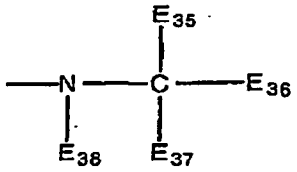


で表される、請求項２に記載の化合物。

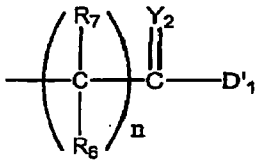
【請求項４】

上記末端分枝基が式：

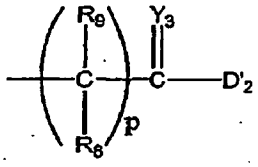
【化８】



[式中、
E_{35}は
【化９】

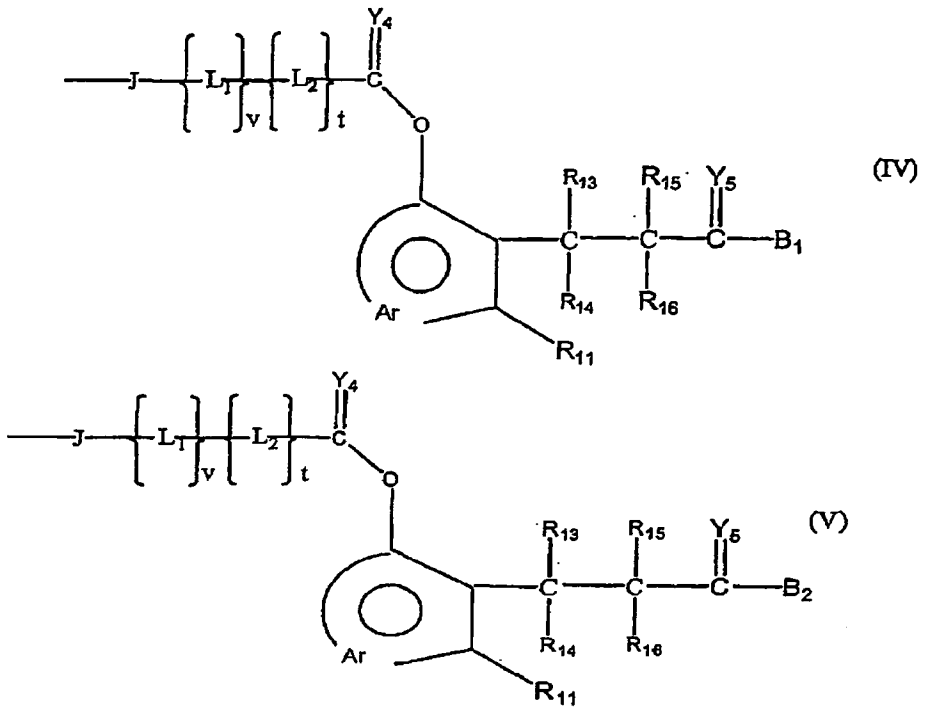


であり；
E_{36-38}は独立に、H、E_{35}または
【化１０】



であり；
(n)および(p)は独立に、0または正の整数であり；
Y_{2-3}は独立に、O、SまたはNR_{10}であり；
R_{6-10}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
D'_1およびD'_2は独立に、OH、

【化１１】

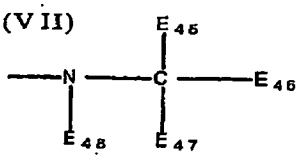


(IV)

(V)

または

【化１２】



(VII)

{式中、

(v)および(t)は独立に、0または約6までの正の整数であり；

L_1およびL_2は独立に選択された二官能性リンカーであり；

Y_{4-7}は独立に、O、SおよびNR_{14}からなる群から選択され；

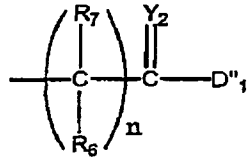
R_{11-14}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；

Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成分であり；

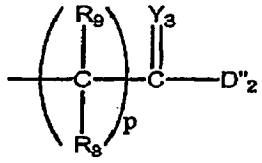
B_1およびB_2は独立に、脱離基、OH、ヒドロキシル基含有成分またはアミン基含有成分の残基からなる群から選択され；

E_{45}は

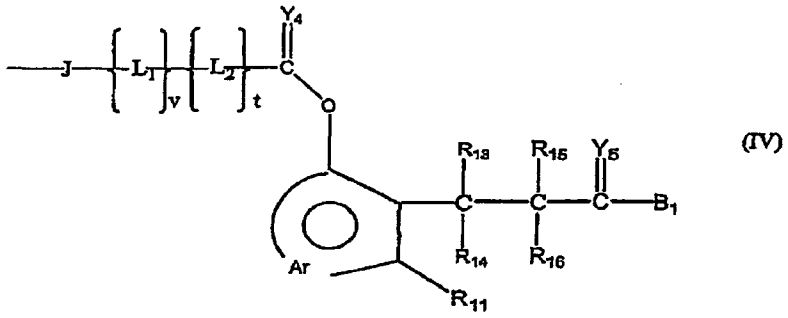
【化１３】



であり；
E_{46-48}は独立に、H、E_{45}または
【化１４】

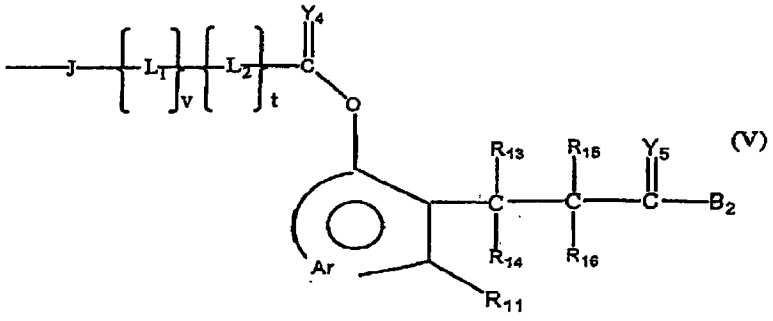


（式中、
D''_1およびD''_2は独立に、OH、
【化１５】



(IV)

または
【化１６】

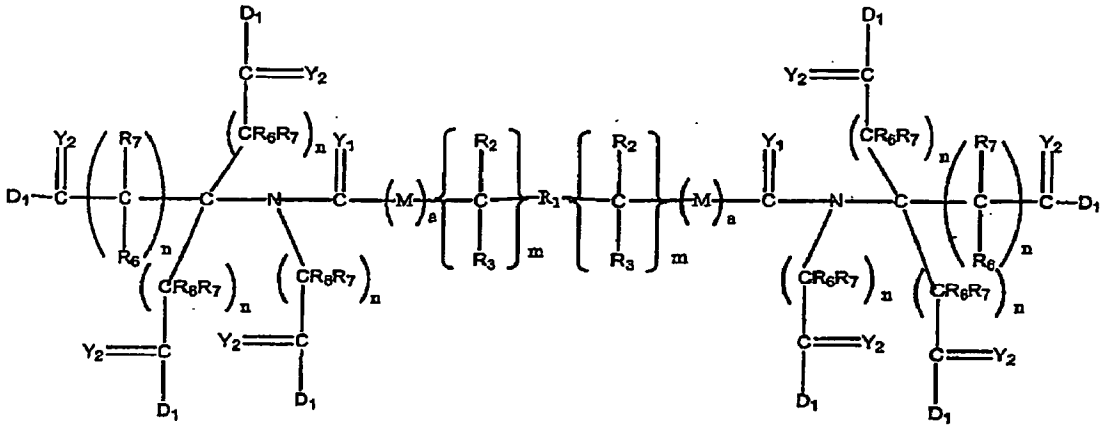


(V)

である）
である｝
である］
で表される、請求項１に記載の化合物。
【請求項５】
Y_1がOである、請求項３に記載の化合物。

【請求項６】
R_1がポリアルキレンオキシド残基を含んでなる、請求項１に記載の化合物。
【請求項７】
R_1がポリエチレングリコール残基を含んでなる、請求項６に記載の化合物。
【請求項８】
R_1がポリエチレングリコール残基を含んでなる、請求項３に記載の化合物。
【請求項９】
R_1が
$-C(=Y_6)-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-C(=Y_6)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-C(=Y_6)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-A$、
$-C(=Y_6)-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-C(=Y_6)-$、
$-C(=Y_6)-Y_7-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-Y_7-C(=Y_6)-$、
$-C(=Y_6)-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{23}-C(=Y_6)-$、
$-(CR_{24}R_{25})_e-O-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-O-(CR_{24}R_{25})_e-$、および
$-NR_{23}-(CH_2)_f-O-(CH_2CH_2O)_x-(CH_2)_f-NR_{23}-$
（式中、
Y_6およびY_7は独立に、O、SまたはNR_{23}であり；
xは重合度であり；
R_{23}、R_{24}およびR_{25}は独立に、H、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され；
eおよびfは独立に、0、1、または2であり；かつ
Aはキャッピング基である）
からなる群から選択される、請求項６に記載の化合物。
【請求項１０】
R_1が$-O-(CH_2CH_2O)_x$を含んでなり、かつxは重量平均分子量が少なくとも約20,000であるような正の整数である、請求項９に記載の化合物。
【請求項１１】
R_1の重量平均分子量が約20,000～約100,000である、請求項３に記載の化合物。
【請求項１２】
R_1の重量平均分子量が約25,000～約60,000である、請求項３に記載の化合物。
【請求項１３】
式

【化１７】

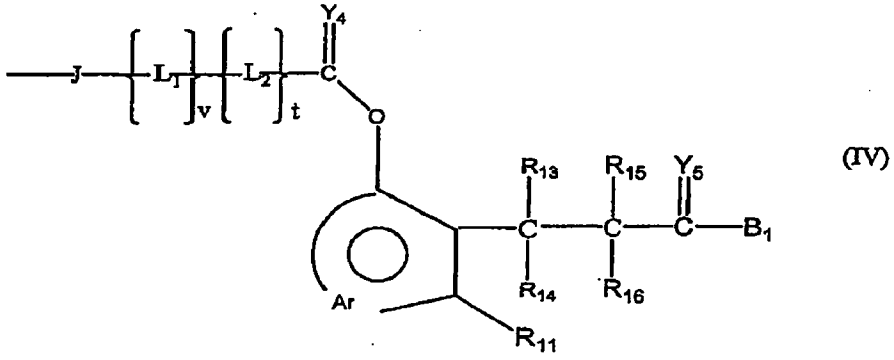


で表される、請求項３に記載の化合物。

【請求項１４】

D_1が

【化１８】



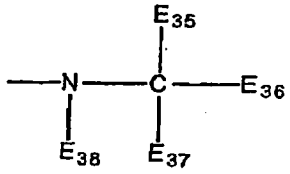
(IV)

である、請求項１３に記載の化合物。

【請求項１５】

D_1が

【化１９】



である、請求項１３に記載の化合物。

【請求項１６】

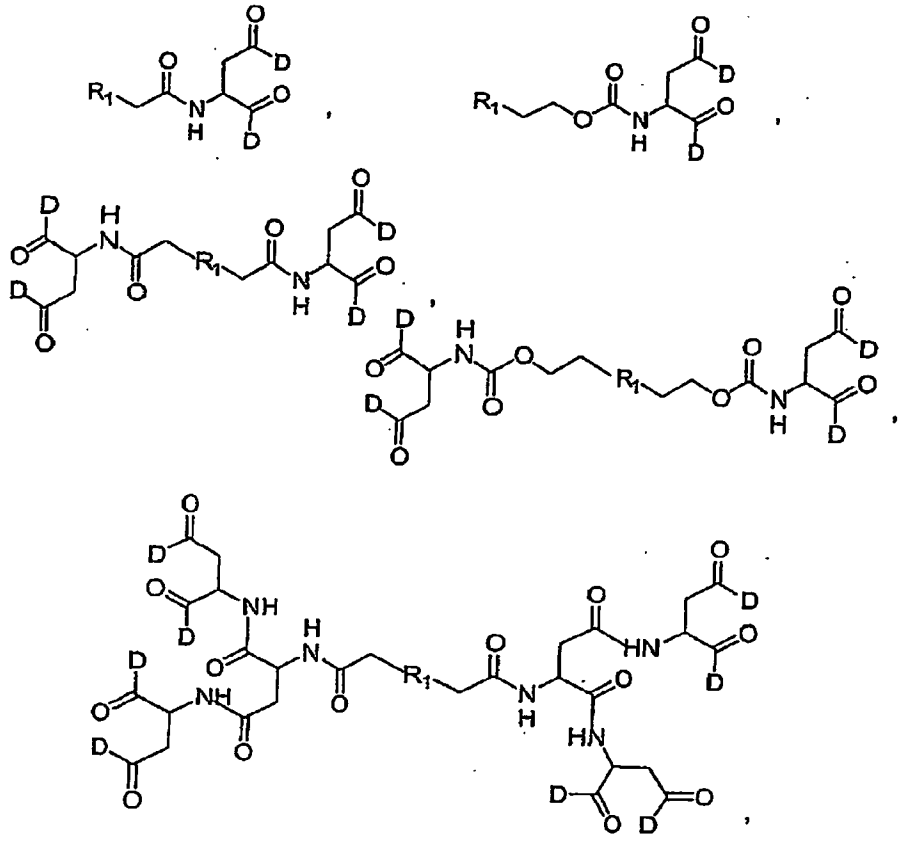
L_1が$(CH_2CH_2O)_2$である、請求項１に記載の化合物。

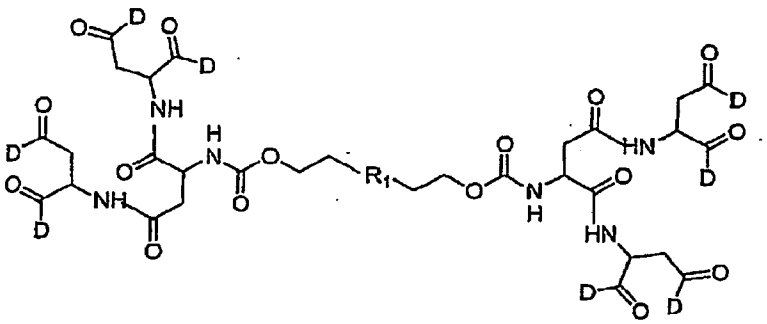
【請求項１７】

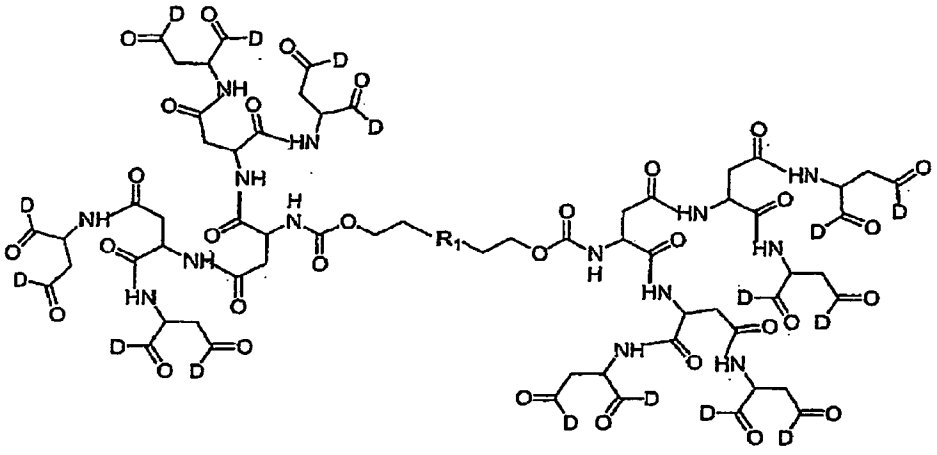
L_2が$-CH_2-$、$-CH(CH_3)-$、$-CH_2C(O)NHCH(CH_3)-$、$-(CH_2)_2-$、$-CH_2C(O)NHCH_2-$、$-(CH_2)_2-NH-$、$-(CH_2)_2-NH-C(O)(CH_2)_2NH-$および$-CH_2C(O)NHCH(CH_2CH(CH_3)_2)-$からなる群から選択される、請求項１に記載の化合物。

【請求項１８】

【化２０】

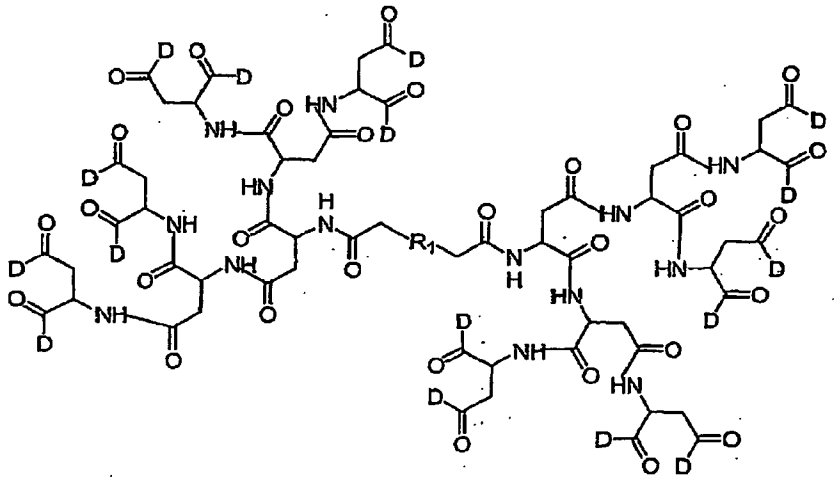






および

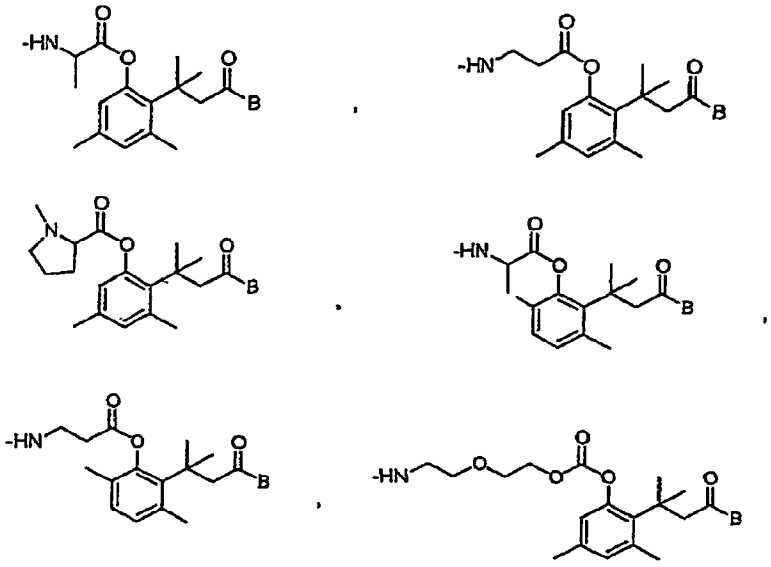
【化２１】



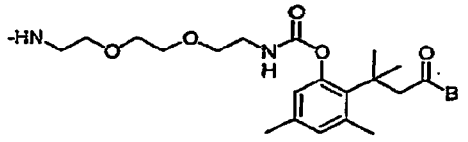
式中、

R_1はPEG残基であり、かつDは

【化２２】

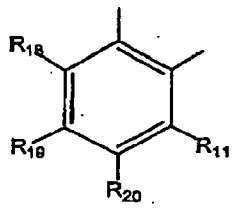


および
【化２３】

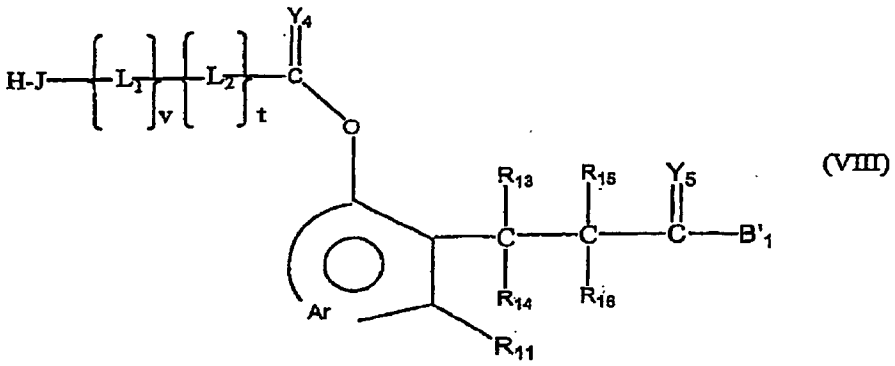


(式中、
Bはアミンまたはヒドロキシル基含有薬物の残基である)
からなる群から選択される|
からなる群から選択される、請求項１に記載の化合物。
【請求項１９】
Bがダウノルビシン、ドキソルビシン；p-アミノアニリンマスタード、メルファラン、Ara-C(シトシンアラビノシド)、ロイシン-Ara-C、およびゲムシタビンからなる群のメンバーの残基である、請求項１８に記載の化合物。
【請求項２０】
治療が必要な哺乳類に投与するための、有効量の請求項１に記載の化合物(式中、D_1は生物学上活性な成分の残基である)を含む医薬組成物。
【請求項２１】
治療が必要な哺乳類に投与するための、有効量の請求項１８に記載の化合物を含む医薬組成物。
【請求項２２】
Arが式：

【化２４】

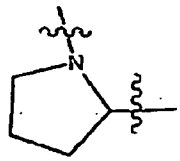


(式中、
R_{11}およびR_{18-20}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択される)
で表される、請求項１に記載の化合物。
【請求項２３】
R_{11}およびR_{18-20}が各々、HまたはCH_3である、請求項２２に記載の化合物。
【請求項２４】
高分子複合体の製造方法であって、
式(VIII):
【化２５】



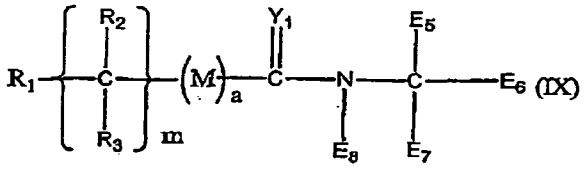
(VIII)

(式中、
(v)および(t)は独立に、0または約6までの正の整数であり;
JはNR_{12}または
【化２６】

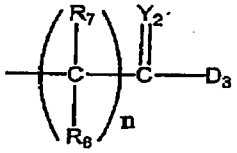


であり;
L_1およびL_2は独立に選択された二官能性リンカーであり;
Y_{4-5}は独立に、O、SおよびNR_{17}からなる群から選択され;
R_{11-17}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択され;
Arは式(I)に含まれる場合に多置換芳香族炭化水素または多置換複素環基を形成する成

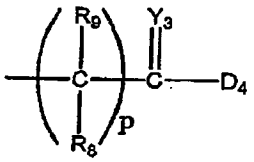
分であり；かつ
B'_1はヒドロキシルまたはアミン基含有成分の残基である）
で表される化合物と、式(IX):
【化２７】



(式中、
E_5は
【化２８】



であり；
E_{6-8}は独立に、H、E_5または
【化２９】



であり；
D_3およびD_4は独立に、OH、保護されていないアミンまたはヒドロキシルと反応しうる脱離基、または末端分枝基であり；
R_1は高分子残基であり；
Y_1はO、SまたはNR_4であり，
MはO、SまたはNR_5であり；
(a)は0または1であり；
(m)は0または正の整数であり；
(n)および(p)は独立に、0または正の整数であり；
Y_{2-3}は独立に、O、SまたはNR_{10}であり；かつ
R_{2-10}は独立に、水素、C_{1-6}アルキル、C_{3-12}分枝鎖アルキル、C_{3-8}シクロアルキル、C_{1-6}置換アルキル、C_{3-8}置換シクロアルキル、アリール、置換アリール、アラルキル、C_{1-6}ヘテロアルキル、置換C_{1-6}ヘテロアルキル、C_{1-6}アルコキシ、フェノキシおよびC_{1-6}ヘテロアルコキシからなる群から選択される）
で表される化合物とを、高分子複合体を生成させるのに十分な条件下で反応させることを含んでなる、上記方法。